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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE FORTY-FOURTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY ITHACA, NEW YORK, SEPTEMBER 8, 9, 10, 1952

Effect of the tobacco mosaic disease on the yield of unstaked tomatoes. ALEXANDER, L. J. Plants of Rutgers, Stokesdale, and a breeding line, 80½, were inoculated with tobacco mosaic virus at 3 different dates in each of 2 years. In 1950, a yellow strain of the virus was used. Some of the plants were artificially inoculated on June 5, others on July 17, and the remainder allowed to become naturally infected on or after August 16 when harvest was initiated. In 1951, a green strain of the virus was used with approximately the same dates of inoculation and harvest. In 1950, where the plants were infected late, the yield was: Stokesdale, 21.83; Rutgers, 15.39; and 80½, 19.69 tons per acre. The yields from the plants inoculated June 5th were reduced as follows: Stokesdale, 50; Rutgers, 52; and 80½, 31 per cent. In 1951, the plants infected late yielded: Stokesdale, 24.87; Rutgers, 18.83; and 80½, 26.66 tons per acre. Early infection reduced the yields as follows: Stokesdale, 30; Rutgers, 15; 80½, 19 per cent. Results indicate that the yellow strain of the virus caused a greater reduction in yield than did the green strain, that delaying the time of infection reduced the loss, and that of the 3 varieties tested, the breeding line, 80½, was less seriously affected by tobacco mosaic.

Factors influencing the incidence of Hypoxylon canker of aspen. ANDERSON, RALPH L. Analyses were made to evaluate the influence of tree vigor, stand conditions, and environment on the incidence of *Hypoxylon* canker on 211 plots of aspen in the Lake States Region. Incidence ranged from 1.8 per cent in northernmost Minnesota to 4.8—6.1 per cent in other areas. No correlation was found between tree vigor or site index and percentage of trees infected, but a given percentage causes greater damage on poor sites. Prevalence is reduced in old stands. A high incidence of infection is associated with low stand density. There apparently is a causal relation, as the distribution pattern of cankers also differs with density of stand. There also is a tendency for cankers to face in a given direction, which varies with locality. The presence of other tree species was associated with high incidence of infection. Analyses of soil and water relations, site history and successional trends did not yield convincing correlations. Poor sites, low stand density, and the presence of other species apparently should be avoided in managing the aspen type.

Laboratory screening of chemicals for control of rice white tip. ATKINS, J. G., JR. AND E. H. TODD. Approximately 50 chemicals were screened in the laboratory as seed treatments for control of the rice white tip nematode, *Aphelenchoides oryzae*. Previous tests had shown that theoretically the disease might be controlled by any treatment eliminating the nematodes from the seed. Rough rice and hulls from infested Zenith rice were treated at high dosages and held in closed containers for at least 48 hours. In rating the materials for nematocidal action hulls were placed in water and examined microscopically for viable nematodes. Laboratory germinations were used to detect phytotoxicity. Materials promising at high dosages were tested at lower dosages. The organic mercury fungicides at dosages necessary to kill the nematodes were too injurious. Most of the fungicides, insecticides and nematocides tested were either ineffective or too phytotoxic. Four experimental chemicals combining good nematocidal action with low phytotoxicity were considered the most promising of all chemicals tested. Experimental Nematocides N-244 and N-245 (10 per cent dust formulations) at 2 to 4 oz. per bushel gave complete or nearly complete nematode kill in several tests with no injury. The promising materials were selected for field testing at various dosages.

Results of crossing isolates of Chalara quercina from different states. BARNETT, H. L. Thirty West Virginia isolates and more than 70 single spore isolates of *Chalara quercina* from 9 states were included in this study. For the most part, isolates were crossed with 4 standard West Virginia isolates of known compatibility groupings. Reciprocal crosses were also made. *C. quercina* is normally heterothallic, bisexual, self-sterile and cross-fertile. When used as female, cultures were grown at 25° C. for 6 days on a dilute maltose-hydrolyzed casein medium, then spermatized with a conidial suspension. Three days later the cultures were examined for perithecia and ascospores. All isolates were capable of acting as male and fell into one of the 2 known compatibility groups. Although the production of perithecia in certain crosses was inconsistent with the normal pattern, no geographic races were found. Variations in color of mycelium and abundance of conidia were evident. Some isolates failed to act as female when crossed with a number of other isolates. One culture, No. 805, was shown to be a unisexual male. From a cross (633, West Virginia dark B ♂ ♀ × 805, Minnesota light A ♂) 23 single ascospore cultures segregated as follows: 14 dark B ♂ ♀, 2 dark A ♂ ♀, 6 light B ♂, 3 light A ♂.

Transmission of a ringspot-virus disease of Syringa vulgaris by grafting. BEALE, J. H. AND HELEN PURDY BEALE. In 1935, an illustrated account of a ringspot virus of lilac in North Bulgaria was published by Atanasoff but no evidence of its transmissibility was presented. A similar disease of the common lilac, *Syringa vulgaris L.* was observed in the vicinity of Yonkers, New York, in 1949 by the authors, who reported it later (Proc. VII Internat. Bot. Congr. (Stockholm) 1950. In press). The senior author has now succeeded in transmitting the disease to 2 varieties of lilac by grafting. An indication of transmission was observed by the junior author recently in 4 different varieties of lilac affected with the ringspot virus at the Arboretum of the University of Wisconsin. All of them had been grafted, prior to their purchase, on diseased lilac, but where healthy understock was used, as in the case of 1 of the varieties, no ringspot symptoms were exhibited.

Growth of Chalara quercina in liquid media as influenced by various vitamins and carbon and nitrogen sources. BECKMAN, CARL H., J. E. KUNTZ, AND A. J. RIKER. *Chalara quercina* H. grew best in liquid media buffered with 12 gr. of mono- and di-potassium phosphate proportioned to give a pH of 4.0 to 5.0. The addition of certain minor elements improved growth. Vitamin deficiencies appeared relatively slight. A mixture of biotin, thiamine, and inositol promoted somewhat better growth than any 1 or combination of 2 of these vitamins. Comparative yields on carbon sources were as follows: Those on dextrin and xylose were best; on maltose, fructose, cellobiose, galactose, glucose, raffinose, mannose, sucrose, and xylan were good; on pectin, arabinose, inulin, melibiose, and cellulose were fair; on rhamnose, starch, and lactose were poor. Among 9 alcohols and 9 organic acids, ethanol and malic acid supported fair growth at low concentrations. Best nitrogen sources of 25 tested were arginine, asparagine, and aspartic acid. Alanine, urea, methionine, and ammonium salts promoted fair growth. Phenylalanine, glycine, and histidine allowed sparse growth, while nitrate salts, isoleucine, threonine, hydroxyproline, tryptophane, tyrosine, cysteine, lysine, and leucine permitted no growth.

Cumulative effects of lime sulfur and Ferrox flotation sulfur on McIntosh apple trees. BURRELL, A. B. Lime sulfur and Ferrox flotation sulfur were applied to 14 single-tree replicates per treatment on a protectant schedule for 10 years in a cool climate. The treatments were: (A) lime sulfur all season, (B) flotation sulfur all season, (C) lime sulfur through petal-fall followed by flotation sulfur, and

(D) flotation sulfur through petal-fall followed by lime sulfur. There was an average of 6.6 applications per year. Slightly better scab control resulted from lime sulfur all season or lime sulfur followed by flotation sulfur. Spray injury to fruit was minor and similar throughout. Yields were lowest with lime sulfur all season, doubtfully better with lime sulfur in late season (treatment D), decidedly better with lime sulfur early followed by flotation sulfur, and best with flotation sulfur throughout. The cumulative yields were in the final order at the end of the third year and were in approximately the final quantitative relationships by the sixth harvest. The treatments had no clear-cut effect on trunk circumference growth, and elongation of terminals was unaffected. With restoration of uniform spraying after the conclusion of the experiment, yields promptly became equal for the 14-tree groups that formerly had received treatments A, B, C and D, respectively.

The boron content of McIntosh apple leaves and fruits in relation to symptoms and methods of application. BURRELL, A. B., DAMON BOYNTON AND A. D. CROWE. Mid-terminal leaves, collected in summer from boron-deficient trees contained 8-18 P.P.M. boron (B), dry weight basis. Leaves from comparable trees that had received 6 annual narrow-ring soil applications of borax or H_3BO_3 contained 29-37 P.P.M. Mature fruits from these untreated trees contained 4-5 P.P.M.; fruits from treated trees, 13-31 P.P.M. Leaves from trees that had received excessive B applications, causing chlorosis, contained up to 200 P.P.M. In 5 Hudson Valley orchards that showed internal cork of fruits in the dry year, 1949, but not since, leaves from untreated McIntosh or Cortland trees contained 14-26 P.P.M. in mid-summer 1950, and 18-28 P.P.M. in 1951. B application to the soil in either fall, 1949, or spring, 1950, caused a small and similar increase in leaf-B. Borax in 2 sprays caused a greater first-year increase but the residual effect was less. Less B was absorbed when broadcast than when restricted to a ring beneath the branches. These differences were more striking in mature fruits than in leaves. Increasing levels of nitrogen fertilization produced corresponding decreases in leaf-B concentration. Among fruit tissues, the endocarp had the highest B concentration, the skin, the fleshy ovary, and the seeds somewhat less, and the floral tube, the least.

Anabiotic survival of a new species of Ditylenchus nematode. CAIRNS, ELDON J. The *Ditylenchus* nematode, a pest in commercial mushroom production, exhibits the phenomenon of anabiosis. The obvious importance of this capacity for survival during periods of adverse environmental conditions warranted qualitative and quantitative study. After being kept in a state of anabiosis for 15 days, very few larvae within the eggs and a reduced number of young hatched larvae were revived. After 40 days in anabiosis, no eggs showed active larvae, and relatively very few second stage larvae were revived. After 100 days in anabiosis, only third and fourth stage larvae and young adults of both sexes were revived, the majority then being fourth stage larvae and adults. Significantly, there is no single life stage contributing individuals capable of anabiotic survival, as in the case of the "Dauer" form in other species and genera. Also, the anabiotic capacity of the early larval stages is noteworthy. Quantitatively, there was a uniform decline in the total numbers of nematodes revived when plotted at time intervals. This nematode has the capacity for reactivation, upon the addition of water, after being in the condition of anabiosis for periods of at least 3 years.

Root necrosis virus of Nicotiana glutinosa. CETAS, ROBERT C. AND A. FRANK ROSS. A previously undescribed virus was discovered during routine tests of stock cultures of potato virus A. In *N. glutinosa* the virus produced grayish-brown, spreading necrotic lesions on inoculated leaves; dark-green veinbanding and necrotic flecks in noninoculated leaves; necrosis of the root and lower stem; and various degrees of stunting. Necrosis of the lower stem appeared to be confined to the cortex and phloem. The virus produced no visible symptoms in Turkish tobacco, vein clearing and vein banding in *N. repanda*, and local lesions on *Physalis floridana* and on *P. peruviana*. *Datura stramonium*, *Gomphrena globosa*, Bountiful and Scotia bean, cowpea, cucumber, pepper, U.S.D.A. Potato Seedling 41956, and zinnia did not become infected following mechanical inoculation. No symptoms were produced on plants of *P. floridana* or of *N. glutinosa* held at 80°F. following inoculation nor was virus recovered from such plants. In juice from infected plants of *N. glutinosa*, the virus was inactivated in 10 minutes at 50°C. but not at 45°C. The dilution end-point was between 1:100 and 1:500. No evidence of cross-protection was obtained between this virus and potato viruses A, X, and Y.

A quantitative electrophoretic study of competition between two strains of tobacco mosaic virus. II. *The physiological age of the leaf as a factor in strain competition in the inoculated leaf.* COHEN, MORRIS, AND ALBERT SIEGEL. Electrophoretic analyses of virus strain mixtures extracted and purified from tobacco leaves previously inoculated with a 50-50 mixture of purified common and mild TMV strains indicate that the physiological condition of the leaf at the time of inoculation influences the degree to which the common strain predominates over the mild strain in those leaves. A single leaf in each of a series of comparable greenhouse-grown tobacco plants about 18 inches tall was inoculated with the 50-50 strain mixture. 4 weeks later, the virus strain mixture extracts of these leaves, which at the time of inoculation were (1) mature, (2) expanding and of medium size, and (3) about half an inch in length, were found to contain, respectively, about 35 to 50, 20 to 30, and 0 per cent mild.

A survey of bacteria antagonistic to Pythium arrhenomanes in Louisiana sugarcane soils. CONNELL, THOMAS D. A survey was made of Louisiana sugarcane soils to determine the numbers of bacteria present and the antagonistic activity of these organisms to *Pythium arrhenomanes*, the causal agent of sugarcane root rot. Soil samples were taken from 13 locations, every 3 months, for a period of a year. The bacterial populations ranged from 9 to 102 millions per gr. of soil, the higher numbers occurring in soils with high moisture contents and neutral to alkaline reactions. Significant differences in bacterial populations were found in soils collected at different seasons, the greatest populations occurring in the spring collections. Bacterial isolates, taken at random from each soil sample, were tested on Czapek's medium for antagonistic activity to *Pythium arrhenomanes*. Of 5638 cultures tested, 202 (3.58 per cent) were antagonistic to the fungus. Most of these were slightly antagonistic, the average inhibition being 5.52 mm. The antibiotic value (mean inhibition in mm. $\times$ the population in thousands per gram of soil) was calculated for each soil tested. In general these values were correlated with the bacterial populations. The highest bacterial populations and antibiotic values were obtained from heavy soils in which root rot is most severe.

Notes on apparently undescribed species of Rhizoctonia on conifer seedlings. COX, R. S. AND W. R. COMEGYS. What is possibly an undescribed species of *Rhizoctonia* was found to cause damping-off of conifer seedlings and to be associated with root-rot and needle blight. Chief distinguishing characteristics of the fungus included sclerotia, growth habit, and color of thallus. Growth is initiated on PDA by the production of 2 types of mycelia: aerial, cottony, and submerged, hyaline. The aerial mycelium collapses with age. About the 5th day, submerged hyphae begin to develop a distinct reddish-brown color, eventually true of the entire colony. Initial crosswalls are laid down seldom farther than 3μ from origin of branching. Hyphae become highly vacuolated with age. The deeply-colored, appressed to submerged, sclerotia are formed through a process of bulbous enlargements and lateral budding of cells of a single hypha, not through an intermingling of separate hyphal tips. Eventually, these enlarged cells mass into a spherical to oblong sclerotium or become chain-like.

Several sclerotia may be formed successively by a single hypha. This process results in a ropy appearance of the thallus and produces a coarse texture of the agar surface. Anastomosis of tips of actively-growing hyphae is common, and what appear to be clamp connections are occasionally found. Various measurements follow: hyphal cells, 3.2-6.2 ×19.5-123.5 μ; sclerotial cells, 8.5-46.5 μ; sclerotia 40-120 × 40-201.5 μ.

Inducing disease resistance with plant growth-regulators. DAVIS, DAVID. Both physical and chemical agents have been reported to alter susceptibility of the tomato plant to *Fusarium* wilt. Plant growth regulators are known to produce marked changes in the biochemical processes of higher plants. A number of growth regulators were applied to tomatoes in the chemotherapy assay for *Fusarium* wilt. For example, a single application of 2,4-D (5 p.p.m.) to foliage 10 or 4 days before or 4 days after inoculation with *Fusarium* resulted in wilt the severity of which was 0, 12 and 78 per cent of that in check plants, respectively. Naphthaleneacetic acid, β-naphthoxyacetic acid, indole-3-acetic acid and 2,3,5-triiodobenzoic acid (an antagonist of indole-3-acetic acid) all reduced disease in the same pattern as that described for 2,4-D. Thus, the effect of treatment always became more pronounced with time. The effect of growth-regulators on carbohydrate metabolism, for example, likewise follows a time pattern. In view of their low fungitoxicity and of the time which must elapse before the maximum effect is attained, these compounds are unlikely to reduce disease by direct action as fungitoxicants or toxin inactivators. Accordingly, it is postulated that these compounds produce metabolic changes in the host, rendering it unfavorable for the development of disease.

Chemotherapy of tobacco mosaic virus. DAVIS, DAVID. Recently Davis and Dimond postulated that 4-chloro-3,5-dimethyl-phenoxyethanol (1182) induced resistance to *Fusarium* wilt of tomato by altering the host's metabolism. A direct correlation was observed between a change in disease susceptibility and the water-soluble nitrogen content of host tissue. The intimate relation between nitrogen metabolism of the host and virus multiplication suggested that 1182 could be used as a chemotherapeutant for the control of tobacco mosaic virus (TMV). The resistance of *Nicotiana glutinosa* to TMV was increased with high significance by 10 daily soil applications of 50 ml. of 16 p.p.m. 1182. The number of local lesions per unit area or per leaf in 1182-treated plants was reduced to approximately 1/3 of the check. This change in susceptibility was independent of leaf age and concentration of virus inoculum. TMV was not inactivated *in vitro* when subjected to 1182 at 200 p.p.m. for 5 days at room temperature. The failure of 1182 to inactivate TMV *in vitro* suggests that the alteration in susceptibility to TMV was caused by a modification of the host's metabolism. Thus, the predicted activity concerning 1182 was verified.

Stem rust control with fungicides. DICKSON, J. G., G. R. GRIMM AND A. L. HOOKER. Certain naphthoquinones and phenols have shown fungicidal value at low concentrations in the control of stem rust of cereals. Urediospore germination of *Puccinia graminis tritici* was prevented at concentrations as low as 2 p.p.m. Spray applications prior to infection using the fungicides with spreader and stabilizer resulted in 90 to 100 per cent rust control at low concentrations for several compounds studied. Spray applications 2 days after inoculating seedlings with urediospores in the greenhouse at 20°C. showed no reduction in subsequent uredial development with the phenols and only small reductions with the naphthoquinones used. Ethyl alcohol solutions of technical 2, 3-dichloro-1, 4-naphthoquinone (Phygon) with a stabilizer appeared to be the most economical compound presently available.

Biochemistry of the lycomarasmin-iron chelated complex. DIMOND, A. E. AND P. E. WAGGONER. The colorless lycomarasmin-iron complex is more toxic to tomato cuttings than lycomarasmin itself. Chelating agents which remove iron from lycomarasmin-complex should diminish its potency. Extent of colored complex formation with synthetic chelating agents showed that a,a′-dipyridyl and o-phenanthrolene remove ferrous iron completely and rapidly from the lycomarasmin-complex. 8-Quinolinol removes both ferrous and ferric iron very slowly and incompletely. As indicated by a,a′dipyridyl, the ferric lycomarasmin system slowly changes to a ferrous-ferric lycomarasmin equilibrium. This system is no more toxic that ferro-lycomarasmin. Both chemical and toxicity assays indicate that chelation occurs between 2 lycomarasmin molecules and 1 ferrous ion. Because toxicity and chelating ability of lycomarasmin are diminished as NH_3 is split by hydrolysis from the amide group, chelation involves bonding to this group. Both chemical and toxicity assays indicate that ferrous or ferric iron partially displace copper from the inactive cupri-lycomarasmin complex. Addition of a,a′dipyridyl or o-phenanthrolene to the lycomarasmin-iron complex also reduced lycomarasmin toxicity. If lycomarasmin is truly involved in the syndrome of Fusarium wilt, cupric ion, a,a′dipyridyl or o-phenanthrolene should act as chemotherapeutants by removing iron from the lycomarasmin complex. Neither glutathione nor cysteine decreased toxicity of the lycomarasmin-iron complex.

Control of damping-off of sugar beet by Bacillus subtilis. DUNLEAVY, JOHN M. Soil samples were collected from eastern Nebraska soils and assayed for bacteria showing antagonism toward a species of *Rhizoctonia* which causes severe damping-off of sugar beet. One species of bacterium which showed considerable antagonism was isolated frequently and identified as *Bacillus subtilis*. The bacteria were grown in a liquid medium for 7 days after which they were centrifuged, washed and recentrifuged 4 times. They were then resuspended and killed by autoclaving twice. When the suspension was added to nutrient agar, *Rhizoctonia* was greatly inhibited. In greenhouse tests living bacteria were introduced into sterile soil. After 7 days *Rhizoctonia* was added to the soil and the soil planted to sugar beets. This treatment resulted in a marked decrease of damping-off in comparison with soil which did not contain the bacteria.

An isolate of Fusarium with unusual virulence for the resistant Iroquois muskmelon. EIDE, CARL J. AND ARMAS MAKILA. Fusarium wilt of muskmelon (*Fusarium bulbigenum* var. *niveum*) occurred in Minnesota in 1951 on the variety Iroquois, which had hitherto been resistant in the field. Some of the isolates from wilted plants proved more pathogenic on Iroquois than on Golden Osage or Pride of Wisconsin, varieties which have always been highly susceptible to common isolates. Wilting of Golden Osage and Pride of Wisconsin also occurred but more slowly than in the Iroquois. Previous work has shown that isolates of the fungus differ greatly in pathogenicity, but the relative pathogenicity has heretofore been the same in different varieties. The race that attacks Iroquois does not differ from common isolates in culture. This therefore appears to be a case of pathogenic specificity in Fusarium on muskmelon.

The role of gliotoxin in the soil. EVANS, ELFED AND DAVID GOTTLIEB. Previous investigations in this laboratory have dealt with the significance of antibiotics as possible factors affecting the general microflora of the soil. The present study is an extension of such work to the neutral antibiotic Gliotoxin. Having tested a number of organisms, maximum yield of the antibiotic was found to be given by *Trichoderma viride* in Raulin-Thom medium in shake flasks after a period of 6 days. Using the conidia of *Sclerotinia fructicola* as test organisms, a spore germination dilution technique was adopted as standard assay. By successive extractions with ethyl acetate and chloroform, it was possible to detect 0.2γ of Gliotoxin per gram of soil. *Trichoderma viride* was found capable of producing Gliotoxin in 6 days after the infestation of sterile soil. In non-sterile soil, however, it appears that this organism is incapable of producing detectable quantities of the toxic material. The antibiotic-producing capacity of *Trichoderma*

viride having been ascertained, pot experiments were set up to see if this organism was capable of controlling the damping-off disease caused by *Rhizoctonia solani*. It was found that the pathogenic organism was controlled to some degree in both sterile and non-sterile soil, when either pea or radish seeds were used as test plants. A study of the effect of both *Trichoderma viride*, and *Aspergillus clavatus* on control of crown gall in raspberries is in progress.

An attempt to concentrate golden nematode-stimulatory elements from potato-root leachings. FASSULIOTIS, GEORGE AND JULIUS FELDMESSER. Potato-root leachings have been separated, on the basis of differential freezing points, into fractions which result in significantly different larval emigration rates when in contact with cysts of the Golden Nematode, *Heterodera rostochiensis* Woll. Leachings maintained at 28° to 30°F for 40 hours resulted in 2 fractions, frozen and unfrozen. The unfrozen fraction caused a greater rate of larval emigration than either the frozen portion, tap water, or unmodified potato-root leachings.

Observations on the nematocidal action of some organophosphorus insecticides. FEDER, WILLIAM A. Free-swimming foliar nematodes were highly refractile to the toxic action of insecticides in which they were immersed. They lived as long in solutions of Parathion, TEPP, Systox and Selenium, as in distilled water. Since some of these materials are nematocidal when sprayed or drenched on nematode infested plants, their mode of action was of interest. To discover if the vapor phase was nematocidal, bits of nematode-infested tissue were placed in a watch glass which was seated in a petri dish on a filter paper disc containing a definite concentration of toxicant. When the dish was covered, the nematodes within the tissues were exposed to the vapor phase of the toxicants but never to the liquid toxicants. After 48 hours incubation in the dark at 25°C. the tissue bits were removed, soaked in water for ½ hour and the number of living and dead nematodes counted. Results with Systox, Parathion, Malathon, EPN, and TEPP indicated that kill was directly proportional to toxicant concentration. OMPA and Selenium (as sodium selenate solution) were not nematocidal in this test, indicating that they apparently do not function as nematocides in the vapor-phase.

Root galls of tomato induced by Heterodera rostochiensis Woll., the golden nematode. FELDMESSER, JULIUS. Root galls induced by *Heterodera rostochiensis* have been observed on the roots of 60 potted tomato plants, variety Bonny Best, grown in sterile soil and exposed to infective larvae of the nematode. Mass root invasion by the larvae occurred on the fifth and sixth days after initial exposure. Localized thickened galled areas were observed on roots 17 to 18 days after invasion. One week later, saccate transparent females were seen protruding from the galled areas. Subsequent examinations revealed galling only in association with the saccate females. In almost all observed instances, the galls occurred so that the female nematodes protruded from that part of the gall distal to the root crown, with the inserted head region pointed toward the root crown. A search of the literature indicates that this is the first report of root galling due to *H. rostochiensis*. The cytology of this host reaction is being investigated. Slides showing the galls and their association with the female nematode have been prepared.

Sources of resistance to two races of the tomato late blight fungus. GALLEGLY, M. E. Isolates of the tomato late blight fungus (*Phytophthora infestans*) were obtained for use in screening tomato accessions for resistance. Isolates were WV-2T and WV-6T from West Virginia; M-6 from Pennsylvania; M-7 from Indiana; and M-13 from Florida. The isolates were cultured on fresh-frozen lima bean agar. 3- to 6-weeks-old plants were inoculated with zoospore suspensions. Inoculated plants were held in a 20° C. temperature-humidity room for 24 hours and then removed to a 20° greenhouse or left in the artificially lighted rooms (with decreased humidity) for 7 days. The plants were then rated from 1-5, immune to susceptible. Preliminary experiments showed accession numbers 19 (ornamental tomato from Florida), 36 (cherry tomato from North Carolina), and 106 (PI 108245) resistant to isolates M-7 and WV-6T, and numbers 36 and 106 resistant to isolate M-6. Resistant plants reacted with specks and small lesions. All were susceptible to isolates M-13 and WV-2T. This indicates that there are at least 3 pathogenic races of the tomato late blight fungus. Other accessions found resistant to isolates M-6, M-7 and WV-6T are numbers 126 (PI 110595), 502 (PI 163246), 700, 701, and 702 (all from Mexico), 819 (PI 112835), and 878 (Jamaca wild from Graham at Ottawa, Ontario.

A new root rot of red clover. GERDEMANN, J. W. A fungus not previously reported has been found commonly associated with red clover root rot in some areas of Illinois. In greenhouse tests it is pathogenic on red clover and alfalfa. When plants are grown in infested soil the fungus enters the roots by direct penetration and causes a brown to black root rot. It also causes damping-off of seedlings. Brown acervuli are borne on suscepts and various kinds of sterilized plant parts. The conidia are hyaline, 1-septate, and have a cilium at each end. Small black sclerotia are produced on suscepts, natural media, and potato dextrose agar. An appropriate genus for this fungus has not been found. The conidia resemble those of *Discosiella*; however, the fruiting body of *Discosiella* is described as a pycnidium. Taxonomically it appears to be closely related to *Pseudodiscosia* differing in number of conidial septations and color of the acervulus.

Septoria black stem of oats in Minnesota. GOTO, S. Black stem of oats caused by *Septoria avenae* has been common in Minnesota for the past few years. The dark brown lesions usually occur on leaves, nodes, and sheaths, and less often on glumes. Smooth black blotches may appear on the parts of the stem and hulls underneath sheath and glume lesions. Seedlings of more than 90 varieties and selections of oats were inoculated in the greenhouse with water suspensions of pycnidiospores. Black streaks appeared in 3 to 4 days, then coalesced within 2 weeks to form the typical dark brown, slightly-haloed lesions. Although some varieties and lines were somewhat resistant, none was immune. Light colored lesions with typical pycnidia and pycnidiospores were observed for the first time in 1951.

Purple leaf spot of orchard grass. GRAHAM, J. H. Purple leaf spot, caused by *Stagonospora maculata*, is present on most orchard grass plants in the Northeast, often killing entire blades and attacking other above ground parts. The disease first appears as tiny watery spots which develop into sub-elongated, purplish-black lesions, 2-4 mm. long. In greenhouse inoculations of 12 species representing 10 genera of forage grasses only *Dactylis glomerata* and *D. aschersoniana* were susceptible. Most isolates produced pycnidia on soybean agar and sterile birdsfoot trefoil stems. Optimum temperature for mycelial growth was approximately 25°C. while best spore germination and infection occurred at 22°C. It was found that penetration and some infection occurred within 6 hours. Penetration also was observed by microscopic examination of stained leaf sections. It is known that a similar environment occurs on many summer nights in Pennsylvania and could account for the increase of disease even during periods of low rainfall. Penetration is primarily direct; appressoria are formed usually between epidermal cells. Primary infection in spring is by pycnospores from dead leaves and culms.

Rod-like particles in extracts from virus-free potato plants. GUTHRIE, JAMES W. Rod-like particles were found in extracts from 80 potato plants of seedling 41956, which is reported to be immune to potato virus X. Attempts to demonstrate the presence of a virus in this variety by both mechanical inoculation and graft unions with tomato, tobacco and *Datura stramonium* have yielded negative results. When plant extracts from seedling 41956 were

combined with extracts from plants infected with potato virus Y or tobacco mosaic and inoculated to tobacco the synergistic effect typical of potato virus X in this combination was absent. Rod-like particles were also found in extracts obtained from 25 virus-free potato plants of the Chippewa variety. Further investigation revealed that similar particles were also found in extracts from 5 varieties of potatoes grown from true seed. These plants were grown in insect-proof cages in steamed soil and sampled when about 5 inches high. All attempts to demonstrate the presence of a virus in these plants were negative. From such evidence it is concluded that the frequency of these rod-like particles in large numbers in extracts in the absence of infectivity seems to indicate that such particles are not necessarily potato virus X itself.

Some fundamental studies on cold-testing of corn. HALTIWANGER, WM. L. AND C. C. WERNHAM. Untreated seed of 6 corn hybrids previously rated for cold tolerance was subjected to 4 species of *Pythium*, *P. arrhenomanes*, *P. debaryanum*, *P. irregulare* and *P. ultimum*, 5 strains of *Gibberella zeae* and 4 collections of *Diplodia zeae*, alone and in all possible combinations and with non-amended checks. Soil from a corn field inoculated 3 successive years, was compared with a sterilized portion of it, washed river sand and vermiculite as germination media, at 45°, 50° and 55° F. for durations of 7, 10 and 14 days. The seed was planted in perforated Dixie cups, inoculum (20 gr.) was added or withheld, and then covered 1.5 inches with medium and watered to field capacity. Two 25 seed replicates were employed. Cups were completely randomized within the refrigerator. Germination means decreased as duration of incubation increased, but was significant only between 7 and 14 days. As temperature increased germination decreased, but was significant only between 45° and 55° F. Non-sterilized soil provided the lowest germination. Media per se had no effect on germination. Organisms were the only variables operating to produce lower germination. *D. zeae* had least effect at the temperatures studied. *Pythium* spp. exhibited little pathogenicity in combination with *G. zeae*. *Pythium* spp. exhibited their greater pathogenicity at 55° F.

Effect of seed treatment on seedling stand of small-seeded legumes in Wisconsin. HANSON, E. W. Field tests made in Wisconsin during 1949, 1950, and 1951 did not, in most cases, indicate any benefits from fungicidal seed treatment of alfalfa, red clover, or sweetclover. No significant increases in stand were obtained from treatment with Arasan, Phygon, or Vancide-51 when the plots were seeded in Almena silt loam, Miami silt loam, Omega loamy sand, or Plainfield sand. Significant increases in stand were obtained from the use of each of the above mentioned fungicides when the plots were seeded in Carlisle muck. The latter soil type, however, is of little importance for forage legume production in Wisconsin. Ceresan, Fermate, New Improved Ceresan, red cuprous oxide, Semesan, Jr., Spergon, and Vasco-4 were tested in the field in Miami silt loam only, where they did not give significant increases in stand. Phygon probably should not be used for treating seed of sweetclover, even though it is a good fungicide, because it tends to cause the seed coats to adhere tightly to the cotyledons thus preventing their opening.

Red clover, a reservoir of legume viruses in Wisconsin. HANSON, E. W., AND D. J. HAGEDORN. An extensive study of the viruses occurring in red clover in Wisconsin in 1949, 1950, and 1951 has shown that this host commonly harbors many viruses. Among those that have been repeatedly isolated from naturally infected red clover are the red clover vein-mosaic virus, the pea common mosaic virus, the bean yellow mosaic virus, the alsike clover mosaic virus, the Wisconsin pea streak virus, and the alfalfa mosaic virus. In addition, several others were obtained which have not yet been identified. During the period of this study, the red clover vein-mosaic virus was the most prevalent, followed by approximately equal amounts of pea common mosaic virus and bean yellow mosaic virus. The Wisconsin pea streak virus and alsike clover mosaic virus were next in prevalence followed by alfalfa mosaic virus. Identifications were based on symptoms, host range studies, and physical property tests. These studies show that red clover serves as a reservoir for many legume viruses, several of which may be very destructive to canning peas.

Evidence of existence of physiologic races of Alternaria solani (Ell. & Mart.) Jones & Grout. HENNING, ROBIN G. AND L. J. ALEXANDER. Evidence of the existence of physiologic races of *Alternaria solani* (E. and M.) Jones & Grout has been obtained. From 180 single-conidial isolates obtained from infected tomato leaves collected in Ohio, 10, selected on the basis of cultural differences, were tested for pathogenicity. Seven of these appeared to be distinct physiologic races. A fragmented mycelial suspension of each isolate was sprayed on approximately 6-weeks-old seedlings of *Lycopersicon* spp. The host seedlings were domestic and wild species of the genus *Lycopersicon*, including *L. esculentum*, *L. pimpinellifolium*, *L. hirsutum* var. *glabratum*, *L. peruvianum* type spp. and var. *humifusum*, and *L. glandulosum*. The host lines were selected on the basis of degree of infection by *A. solani* in field plots at Wooster, Ohio. After inoculation the plants were placed in a humidity chamber for 48 hours, and then on greenhouse benches for 8 days, after which notes were taken. The degree of infection was determined by the average number of lesions per inoculated leaflet. This number varied from none to many (5 or more). Three types of lesions occurred: small, restricted, necrotic lesions; larger, spreading, water-soaked lesions; and an "intermediate" type, resembling the small, necrotic lesions, but spreading into the leaf veins. *A. solani* was isolated from all 3 types.

Growth and virus activity in tobacco tissue cultures with naphthalene acetic acid or tryptophan. HILDEBRANDT, ALBERT C., A. J. RIKER AND JEAN L. WATERTOR. *Nicotiana tabacum* tissues of crown gall origin and containing tobacco mosaic virus grew vigorously *in vitro* on a synthetiec medium. It contained only inorganic salts, thiamine, glycine, sucrose, and agar. Subcultures which were made at 6 week intervals during 2 years showed no diminution of virus activity. In these studies, growth was measured as the wet weight. Virus activity was measured weekly for 6 weeks by use of a local lesion test. Media with naphthalene acetic acid at 10^{-7}, 10^{-5}, and 10^{-3} gm. per liter, respectively, supported growth equal or superior to that of control cultures, but 10^{-1} gm. per liter inhibited growth. The number of lesions at the first 3 concentrations was similar to or slightly greater than that of the control cultures, while at 10^{-1} gm. per liter the number was slightly less. Tryptophan at 0, 0.4, 4.0, 40, 400, or 4,000 mgm. per liter, respectively, progressively inhibited growth with little or no growth at the highest concentration. Virus activity of the cultures was little affected by the 4 lowest concentrations, but at 4,000 mg. per liter where the host growth was negligible, the number of lesions produced was reduced 30 to 50 per cent of that of control cultures.

Inheritance of resistance to tobacco-mosaic disease in tomato. HOLMES, FRANCIS O. In earlier work a tendency of tomato not to become infected by tobacco-mosaic virus was demonstrated in plants derived from hybrids between *Lycopersicon esculentum* and *L. chilense*. The mode of inheritance of this characteristic was not determined because of the high degree of sterility in the hybrid lines. More recently, fruitful lines of tomato resistant to tobacco-mosaic disease were received from Dr. W. A. Frazier. These were characterized by a similar tendency to escape infection. In a series of backcrosses to susceptible lines, segregation occurred among the progeny in the ratio of 260 resistant to 257 susceptible individuals. This approximate 1:1 ratio suggested that the tendency to escape infection by tobacco-mosaic virus was dependent on a single dominant gene.

Further studies in the physiology of Phytophthora phaseoli. Hyre, R. A. and R. S. Cox. Studies were made on the effect of relative humidity and temperature on longevity of sporangia of *Phytophthora phaseoli;* and, on the effect of temperature on germination of sporangia and zoospores and on germ tube growth of zoospores. In the longevity studies, sporangia were placed on dry cover slips which were then suspended for various periods over saturated salt solutions in half-pint jars immersed in a constant water bath (± 0.1°C.) after which the sporangia were incubated at 15°C. in sucrose-potassium citrate solution to determine viability. At 20°C., viability was: 37 per cent after 27 hr. at 100 and 95 per cent relative humidity; less than 10 per cent after 4 hr. at 90, one hr. at 86, and 15 min. at 81 per cent relative humidity. At 30°C., viability was: less than 10 per cent after 27 hr. at 100, 10 hr. at 97, and 2 hr. at 93 per cent relative humidity. For germination studies, the glass slide technique was used. After 24 hr. at 10°, 15°, 20°, 25°, and 30°C., per cent indirect sporangial germination was 90, 88, 20, 1, and 0; per cent zoosporial germination was 16, 77, 87, 83, and 64; and average zoospore germ-tube growth (mm) was 62, 79, 99, 114, and 19 respectively.

Significance of physiologic races of Phytophthora fragariae Hickman in breeding strawberries for resistance to the red stele disease. Jeffers, W. F. and D. H. Scott. By differential varietal response of strawberries growing in red stele infested soils, it can be shown that physiologic races of *Phytophthora fragariae* Hickman occur. These races differ markedly in their ability to produce the red stele disease in various selections and varieties of strawberry. At least 3 such races occur in Maryland. By testing the susceptibility of 14 varieties and selections to 5 isolates of the red stele organism obtained from England, it was shown that at least 4 physiologic races were present. One or more of these is different from races found in Maryland. Originally, breeding for resistance to red stele was thought to be relatively simple since only 1 race was assumed to be involved. However, the finding of several physiologic races greatly complicates this problem. It has been possible to incorporate resistance to several races of the red stele organism into some selections by crossing plants showing resistance to single races. Selections having such resistance together with good quality fruit and foliage that is resistant to leaf spot, leaf scorch, and powdery mildew have been distributed to growers for testing.

Control of root rot of corn under greenhouse conditions by microorganisms antagonistic to Pythium arrhenomanes. Johnson, Leander F. Fungal and actinomycete isolates exhibiting varying degrees of antagonism to *Pythium arrhenomanes* in agar cultures were tested for their ability to reduce Pythium root rot of corn under greenhouse conditions. Isolates tested included species of *Streptomyces, Penicillium, Aspergillus, Spicaria, Fusarium, Gliobotrys, Monilia,* and *Monosporium.* Plate cultures of 37 antagonistic actinomycete isolates and 53 antagonistic fungal isolates were mixed with soils which had been previously sterilized and infested with *Pythium.* One week later corn was planted and the roots were examined after 2 weeks of growth. Seven actinomycetes and 5 fungal isolates significantly reduced the severity of Pythium root rot. The fungal isolates which gave significant control of the disease were species of *Spicaria, Penicillium,* and *Aspergillus.* With few exceptions, the isolates which produced significant control were also highly antagonistic to *Pythium* in agar cultures, producing a zone of inhibition of 10 mm. or more. However, many highly antagonistic isolates did not produce a reduction in root rot severity.

Distribution of wood giving a positive reaction to the acid test for phony in graft-inoculated peach trees. Ken-Knight, Glenn. Small peach trees, from seed planted February, 1948, under screen at Fort Valley, Georgia, were inoculated either with a phony-infected scion applied terminally or with a section of phony-infected root grafted to the tap root in October, 1948. The trees were lifted during March and April, 1950, dissected, and sections taken at 5 to 15 cm. intervals, hydrochloric-acid tested. Of 12 test trees with living scions, 9 gave positive reactions, 2 questionable, and 1 negative. In reacting trees 100 to less than 5 per cent of the stem wood gave a positive test. Of 12 test trees on which the scion died, 2 gave positive reactions, 3 questionable, and 7 negative. Five noninoculated trees gave negative reactions throughout. In 2 of 6 root-inoculated trees all stem wood, and in 3 a portion of it gave positive reactions. In 1 all stem and root wood was negative although the implanted root was alive. Positive reactions in roots were regarded as unsatisfactory because of the prevalence of nematode and crown gall injury. In these incubation-stage trees, positively-reacting wood was generally less widely distributed in roots than in stems. However, a negative reaction does not mean absence of the virus.

Alfalfa seed loss due to Ascochyta imperfecta Pk. (blackstem). Kernkamp, M. F., and G. A. Hemerick. Blackstem caused by *Ascochyta imperfecta,* is one of the most destructive diseases of alfalfa in Minnesota. It attacks all parts of the plant, including the inflorescence, where it is particularly destructive to seed. It may attack the young pod directly and infect the seeds or girdle the peduncle or pedicel and prevent seed development. Infected seeds are lightweight, dark, and shrivelled. Infection of the seed pod results in non-viable seeds or viable seeds of normal size which produce spotted, distorted, or necrotic seedlings. Greenhouse and field experiments, surveys of commercial seed fields, and laboratory analyses of seeds indicated that seed loss is proportional to the amount of the disease. Infestation of viable seeds was proportional to the amount of seed loss. Degree of infestation was determined by number of seeds developing pycnidia on wet blotters in 7 days. Seed losses were determined by the number of shrivelled seeds in a sample. No normal seed was produced by plants inoculated in the greenhouse, 100 per cent being infected. Seed losses in commercial fields ranged from 2 per cent where there was little black stem to 28 per cent in severely infested fields, and infestation of viable seed ranged from 16 to 64 per cent in the same fields.

The effect of copper on growth requirements of Coniothyrium fuckelii. King, T. H., Norman E. Krog and Harry W. Schroeder. Three monoconidial isolates of *Coniothyrium fuckelii* were heterotrophic for thiamin and 2 of them were partially heterotropic for biotin and i-inositol. Parallel series were grown on copper-containing and copper-free medium. Growth rates for isolate 1 were identical in both series when supplied thiamin and kept in light. In darkness growth was normal only when the isolate had been grown on a copper medium previously. On the medium containing biotin, thiamin and i-inositol, however, growth was normal in darkness whether or not previously grown on copper. The growth of isolates 1 and 2, from copper and non-copper series, were compared on 12 media, each containing a different source of carbon. The dry weights of normal isolates 1 and 2 were significantly greater on 9 and 3, respectively, of the 12 carbon sources than when grown previously on copper.

The incidence of Fusarium- and Rhizoctonia-infected plants in wheat, corn, and oats. Kommedahl, Thor. Fusarium-infected plants of corn, wheat, and oats were found more frequently in 8- to 10-weeks-old plants than were found in 4- to 6-weeks-old plants, in experiments made in greenhouse beds of untreated soil. The number of Rhizoctonia-infected plants remained unchanged with age, in corn and oats, but was reduced in wheat. When corn, wheat, and oats were replanted in the soil previously sown to each of these 3 crops, the number of Fusarium-infected plants was approximately doubled and the number of Rhizoctonia-infected plants was correspondingly reduced. Corn interplanted with wheat resulted in a higher frequency of Fusarium-infected plants in both crops than was found in either crop grown separately, or, when either corn or wheat was interplanted with oats. The number of

Fusarium-infected plants in oats was higher when oats were interplanted with corn or wheat than it was in oats grown alone. The number of Rhizoctonia-infected plants was not influenced by interplanting. Upon burying green corn stubble in soil, the number of Fusarium-infected plants was low in corn and oats, but high in wheat. However, when well-rotted corn stalks were buried in soil, the number of Fusarium-infected plants increased in all 3 crops. On the other hand, when corn stalks in any form were buried in soil, the number of Rhizoctonia-infected plants was lowered in wheat but was not affected in corn and oats.

Infectivity of dried inoculum of some foliar pathogens. KREITLOW, K. W. AND HELEN S. SHERWIN. Since pulverized dried grain inoculum of *Sclerotinia trifoliorum* and *Fusarium* spp. offered certain advantages over other methods for screening large numbers of clover plants for disease resistance, tests were made to determine if the dried grain method could be adapted for preparing inoculum of certain foliage pathogens. Cultures of *Rhizoctonia* spp., *Cercospora sojina, Corynespora cassiicola* and *Sclerotinia homeocarpa* were grown on wheat-oat mixture, dried at room temperature, ground and stored at 5°C. Host plants were inoculated by dusting the finely pulverized inoculum over moistened leaves. Although initial infection in some cases was slower than with macerated mycelium sprayed on leaves, the ultimate severity of infection was about the same. Excellent infection was obtained in the greenhouse with dried inoculum of *Rhizoctonia* on *Lotus* spp. and *Bromus inermis;* with *Sclerotinia homeocarpa* on numerous species of grasses, and with *Cercospora sojina* and *Corynespora cassiicola* on soybean in both greenhouse and field.

Arasan for smut control in set-onion plantings. LARSON, R. H., AND J. C. WALKER. Set-onion seed treatment tests were conducted in commercial fields on the upland soils of southeastern Wisconsin, where onion smut (*Urocystis cepulae*) has increased to such an extent that failure to use the formaldehyde-drip treatment has resulted in very serious yield reductions and in oversized sets. In this culture about 65 pounds of seed are planted per acre. Over a 5-year period (1947 to 1951, inclusive) an average of 77 per cent of the plants grown from untreated seed was diseased. With the standard commercial formaldehyde-drip treatment, the average percentage of diseased plants was 6.4. When 1/10 pound of Arasan (tetramethyl-thiuram-disulphide) per pound of seed was used as a dry seed treatment without sticker, the average incidence of smut was reduced to 3.3 per cent. Decrease in disease to an average of only 11.6 per cent resulted when 1/10 pound of Arasan was applied with sticker (Methocel-Dow methyl cellulose). In commercial practice the dry seed treatment of 1/10 pound of Arasan per pound of seed without sticker is more effective than the formaldehyde-drip treatment, and much easier to apply. It has entirely replaced the formaldehyde-drip treatment as an effective control for onion smut in set-onion plantings on the heavily infested upland soils of southeastern Wisconsin.

Seed treatment experiments with helixin B. LEBEN, CURT AND D. C. ARNY. In greenhouse tests, Vicland oat seed naturally infested with *Helminthosporium victoriae* was treated with the antibiotic helixin B (1.8 units per mg.) at an approximate concentration of 0.3 oz. per bu. and with Ceresan M dust (7.7 per cent ethyl mercury *p*-toluene sulfonanilide) at 0.5 oz. per bu. In 3 replicated tests run at different times, the percentages of plants showing seedling blight were: helixin B, 0.6, 1.5, and 4.8; Ceresan M, 7.0, 4.0, and 3.0; untreated, 39.2, 27.7, and 39.4. In the first test emergence was inhibited by the antibiotic treatment. This was due to contact with ethanol, the solvent in which the antibiotic was carried. In the second and third tests the ethanol was allowed to evaporate soon after treatment, and emergence was not inhibited. In spore germination tests with *Glomerella cingulata,* helixin B was active across an air space.

Host preferences of the recently discovered tobacco cyst nematode, Heterodera SP. LOWNSBERY, B. F. In September, 1951, a new cyst-forming nematode was found on tobacco in Connecticut. Reproduction of the tobacco cyst nematode occurs consistently on several tobacco varieties and less readily on tomato. Second stage larvae will attack potato lightly, but examination of stained roots has revealed no maturation in potato. This contrasts with the published host preferences of the golden nematode, *Heterodera rostochiensis* Wollenweber, which morphologically resembles the tobacco cyst nematode. The golden nematode has been found to reproduce consistently on potato, less readily on tomato, and not at all on tobacco. The differences in host preference indicate at least an intra-species difference between the tobacco cyst nematode and the golden nematode.

Fungi isolated from sugarcane soils of Louisiana and their antagonistic effect on Pythium arrhenomanes. LUKE, HERBERT H. Louisiana sugarcane soils include 4 major types. These soils were sampled at 3 month intervals for a period of 1 year, and fungal populations, pH. values, and moisture percentages were determined. A total of 5,218 fungal isolates (approximately 100 from each soil sample) were tested for antagonism to *Pythium arrhenomanes,* the causal agent of sugarcane root rot. Approximately 17 per cent of the isolatets tested were antagonistic to this pathogen in agar cultures. The antibiotic value (the average distance of inhibition in millimeters × number of thousands of fungi per gr. of soil) was used as a measure of the antibiotic activity of the fungi in these soils. A correlation was found between soil type and antibiotic value. Antibiotic values were higher in heavy clay soils than in light sandy soils. However, root rot was not prevalent in sandy soils but was severe in heavy clay soils.

A comparison of Helminthosporium sativum and H. victoriae. LUTTRELL, E. S. A comparative study was made of the morphology and pathogenicity of isolates of *H. vativum* and *H. victoriae* from various cereals and grasses. By inoculations on Victor Grain oat seedlings the isolates could be divided into 2 groups: those causing severe blight of Victor Grain oat (*H. victoriae* group) and those failing to infect this host (*H. sativum* group). The isolates in each of these groups could be further subdivided on the basis of infection patterns produced on a series of differential cereal hosts. Isolates in the *H. victoriae* group tended to produce smaller, paler, thinner-walled conidia, in this respect resembling *H. setariae* more closely than *H. sativum.* However, the question might be raised as to whether these differences are great enough to necessitate the recognition of distinct species or whether it would be more convenient to treat this complex of variable individuals as a single species comprising a number of physiologic races.

Relative humidity in relation to the retention of viability of larvae enclosed in cysts and free larvae of the golden nematode, Heterodera rostochiensis, Wollenweber. MAI, W. F. AND JOYCE VON MECHOW. Larvae enclosed in cysts were stored for periods of time varying from 90 to 327 days at the following relative humidities: 100, 98.2, 94.8, 91.2, 74.6, 49, 25, 10.5, 4.5, and 1.5 per cent. Loss in viability of enclosed larvae was considerably higher at 94.8 and 1.5 per cent than at any of the other relative humidities. This occurred in 3 separate experiments. The losses in viability at the 2 relative humidities occurred among enclosed larvae stored at room temperature and at 75° F., but were not evident among larvae stored at 40° F. A higher death rate at 94.8 and 1.5 per cent was evident after 90 days and all larvae were dead after 190 days. Enclosed larvae kept at 40° F. at 1.5 per cent relative humidity were dead after 327 days. Viabilities were determined in 3 ways: (1) examination of larvae under a microscope; (2) emergence of larvae from cysts exposed to potato root leachings; and (3) number of individuals developing on potato roots. One hundred per cent mortality occurred when free larvae were stored at 100, 94.8, 49, 10.5, and 1.5 per cent relative

humidities at room temperature, 75° F., and at 40° F. for 243 days.

Recovery of aster-yellows and corn-stunt viruses from nonvector leafhoppers. MARAMOROSCH, KARL. Aster-yellows and corn-stunt viruses were introduced by feeding and by injection into the leafhoppers *Dalbulus maidis*, *Macrosteles divisus* and *Agallia constricta*. Recovery of virus was attempted in the following manner. Inoculated nonvector insects were macerated, diluted with saline to 1:100 and the suspension centrifuged. The supernatant fluid was injected into virus-free leafhoppers of respective vector species. No virus was recovered from nonvector species inoculated by injection, although the same inoculum rendered vectors infective after due time. When *D. maidis* and *A. constricta* were permitted to feed for 2 days on diseased asters, virus could be recovered 19 days after they were removed but not immediately after the feeding. Similarly, when *M. divisus* was fed on stunted corn, corn-stunt virus was recovered after 19 days but not after 2.

The comparative effects of tribasic copper sulfate and of ziram on Colletotrichum phomoides in culture. MARSHALL, N. L., AND C. E. COX. Tribasic copper sulfate and ziram were added in a range of dosages to liquid cultures of *Colletotrichum phomoides*. Daily measurements of the growth of the fungus were made by centrifugation of the cultures. Five times more tribasic copper sulfate than ziram was required to prevent growth of this fungus for 1 week. An extra-cellular, colloidal, organic material was elaborated in cultures of *C. phomoides* containing tribasic copper sulfate in concentrations which retarded but did not prevent growth. This material was not formed in treated cultures in which no growth occurred, in untreated cultures, in flasks containing only fungicide and nutrient solution, or in tribasic cooper sulfate-treated cultures of several other fungi. It may be involved in the detoxification of tribasic copper sulfate and thus, may be a factor in the higher tolerance of *C. phomoides* to this fixed-copper fungicide.

The effects of temperature and humidity on the longevity of conidia of Chalara quercina. MCLAUGHLIN, W. D. AND R. P. TRUE. Chips of oak twigs known to contain *Chalara quercina* and glass beads dipped in a conidial suspension of the same fungus were placed in desiccators maintained at relative humidities of approximately 93 per cent, 81 per cent, 55 per cent and 20 per cent, and at temperatures of 30°, 25°, 20° and 10° C. for each humidity. Samples were removed at intervals and tested for viability by placing them on agar plates. Conidia on beads lived longer than those in naturally infected wood chips. Conidia stored at 30° lived less than 16 days at all humidities. At 25° conidia lived less than 30 days except at 20 per cent relative humidity. At 20° conidia were alive in some samples after 111 days and at 10° after 173 days.

Control of damping-off fungi with halogen substituted nitrobenzenes. MILLER, HAROLD J. Pea seed treatment tests with 2,4-dinitrofluorobenzene in greenhouse soil heavily infested with damping-off organisms gave emergence equal to standard seed treatment chemicals at the rate of 0.25 per cent by weight of seed of a 10 per cent active powder formulation. Untreated pea seed gave as low as 0.0 per cent emergence in this soil under similar conditions. Broadcast applications of 10 to 20 pounds per acre of this compound as a spray (emulsion or wettable powder) gave as high as 70 per cent emergence of pea seedlings as compared with only 22 per cent emergence from the untreated areas. No injury was observed to seed planted before or from 2 to 8 days after the soil treatment. None of the related compounds or standard organic fungicides tested gave comparable control when applied to the soil. Control of anthracnose of cotton seedlings with a dosage 0.15 per cent by weight of a powder containing 10 per cent of 2,4-dinitrofluorobenzene was equal to that obtained by other standard seed treatments.

Control of the sting nematode on peanuts in Virginia. MILLER, LAWRENCE I. The association of a stunted plant disorder of peanuts with the external root feeding of the sting nematode (*Belonolaimus gracilis Steiner*) was first noted in 1949. Although definite proof has not been obtained to show that this nematode is responsible for the stunting, the injury has never been observed where the nematode is not present in the soil. Tests conducted over a period of 4 years have shown that a mixture containing 40 per cent by weight of ethylene dibromide (Dowfume W-40) at 10 gal. per acre, applied broadcast with a shank applicator at an 8-in. depth, will effectively control the sting nematode and correct the stunted plant disorder. Fumigation should precede planting by at least 2 weeks. The benefits from the control of the sting nematode have been highly profitable in yield and quality of hay and nuts. Erect-growing varieties, as a class, are more subject to injury than prostrate-growing types. Several collections of bunch, runner, and Spanish varieties of peanut, with good agronomic characteristics, have shown a relatively high degree of tolerance to injury associated with the root feeding of *B. gracilis*. The plowing under of green cover crops prior to planting also reduces the severity of the disorder.

True dosage received by fungus spores treated with labeled 2-heptadecyl-2-imidazoline. MILLER, LAWRENCE P., AND S. E. A. MCCALLAN. Although the ED50 for spores of a number of species of fungi for 2-heptadecyl-2-imidazoline (glyoxalidine), determined by the usual methods, is from about 2 to 10 p.p.m., the true ED50 based on the quantity taken up by the spores, is about 1000 times greater. Moreover, spores of many species take up the required quantity extremely rapidly from dilute solutions. When 10 mg. of spores of *Neurospora sitophila* are exposed to 10 ml. of a solution containing 7 micromols per liter (2.16 p.p.m.), with a possible true dosage of 2160 p.p.m., 70 per cent of this quantity will be taken up in 15 seconds. If the quantity of spores is reduced to 1/10 this amount, a dosage of 6000 p.p.m. is reached in 30 seconds, and 14000 p.p.m. in 5 minutes. Results of attempts at elution suggest true absorption is involved, rather than adsorption. These findings, together with those obtained with sulfur and ferbam, and with copper by Marsh, suggest caution in comparing orthodox dosage response curves obtained with various chemicals, since marked accumulation may occur with some and not with others. These investigations were supported by the Atomic Energy Commission.

Influence of virus upon the growth of one year old sour cherry nursery trees. MILLIKAN, D. F. AND A. D. HIBBARD. A 2-year study has been completed comparing sour cherry budded from ring spot free sources with those from ordinary nursery run sources. In 1950, 3 different combinations of Montmorency on Mahaleb understock were compared, consisting of nursery run on nursery run, ring spot free on nursery run and ring spot free on ring spot free. Bud take averaged 43.7, 51.5 and 61 per cent respectively while the average terminal growth was 40.6, 41.9 and 50.9 in. In 1951, Montmorency trees propagated from buds selected at random from nursery run sources were compared with those propagated from the certified scion block. Bud take percentage for the nursery run trees averaged 65.6, while that for the certified trees was 67. Terminal growth for the certified trees averaged 50.2 in. contrasting with 42.6 in. for the nursery run material. In 1952, a study was initiated to determine the importance of ring spot alone, comparing the growth of ring spot free Early Richmond with the same clone artificially inoculated by strains of known virulence.

Interaction of strains of necrotic ring spot virus. MOORE, J. DUAIN AND D. A. SLACK. Rapidly growing Montmorency sour cherry trees showing striking symptoms of recurrent necrotic ring spot and known to be infected also with cherry yellows virus, but not prune dwarf virus, were inoculated with buds from 4 sour cherry and 4 peach sources known to contain latent necrotic ring spot, cherry yellows, and prune dwarf viruses. Each of the 8 sources was used separately, and the effects produced fell into 4 groups: (a) trees inoculated with buds from 3 cherry sources exhibited shock symptoms on young leaves, and subsequent new leaves were symptomless; (b) inoculations from 1 peach source did not produce shock symptoms until the yr. after inocu-

lation; (c) inoculations from 1 cherry and 2 peach sources caused no shock symptoms, but subsequent inoculations to Montmorency with buds from all trees inoculated with the 6 sources in groups (a) and (c) failed to incite recurrent necrotic ring spot symptoms; (d) inoculations from 1 peach source produced no shock symptoms and had no apparent effect upon the recurrent necrotic ring spot virus which was readily transmitted back to Montmorency from trees budded with this source. All control trees remained unaffected. Data on prune dwarf virus transmission are not yet available.

The cause and transmission of blue dwarf and red leaf of oats. MOORE, M. B. Blue dwarf, previously unreported, appeared in 1951 in Minnesota and neighboring states. Affected plants are dwarfed, uniformly dark bluish-green, and extraordinarily long-lived. The leaves, especially the flag leaf, are shortened and rigid. In red leaf an orange to reddish color appears on the leaves, at first as blotches but later involves the entire leaf progressively from the tip downward. Plants often die prematurely. Blue dwarf plants brought to the greenhouse from the field eventually developed red leaf symptoms also. Aphids from these plants were transferred to oat seedlings and allowed to feed from 1 to 10 days. Red leaf symptoms appeared 16 days after the earliest feeding. No symptoms appeared following feeding by aphids not previously fed on diseased plants. Later, in early spring tests, blue dwarf symptoms appeared together with the red leaf symptoms. *Rhopalosiphum prunifoleae* and *Macrosiphum granarium* transmitted both diseases. The virus nature and aphid transmission of red leaf seems certain, and that of blue dwarf probable. Neither disease has been studied in the absence of the other. All attempts to transmit these diseases manually failed.

Mercury bichloride solution as a surface disinfectant for cereal seeds. MOORE, M. B. AND CHARLES R. OLIEN. Cereal seeds treated with mercury bichloride solution 1:1000 carried a mercury residue that could not be removed with the usual after-rinses with water or sodium hypochlorite, even with a 24-hour wash in distilled water. It can be removed or inactivated, however, by a 5-minute dip in M/5 sodium thiosulfate solution. As a mercury residue may have differential effects on the growth of various species of fungi from cereal seeds, this should be considered in determining microflora of seeds by isolation methods. The residue was detected by the formation of inhibition zones when the seeds were planted in bacteria-seeded potato-dextrose agar plates and by its retarding effect on the growth of fungi from naturally-infected seeds. *Helminthosporium* and *Alternaria* spp. are among those fungi that tolerate a mercury residue quite well.

A new serological test for tobacco mosaic virus. MOORHEAD, ELLEN L., AND W. C. PRICE. A serological test in which sheep red blood cells were incorporated into mixtures containing tobacco mosaic virus (TMV) and its specific antiserum will be described. Purified virus preparations were injected intraperitoneally and intravenously into rabbits over a 6-week period to induce antibody formation. 0.1 ml. of 2-fold serial dilutions of virus and 0.1 ml. of 2-fold serial dilutions of antiserum were mixed with 0.2 ml. of a 1 per cent solution of freshly drawn, washed, sheep red blood cells and allowed to stand for 3 hours at 5° C. In negative reactions, the red blood cells settled, forming a small red button at the bottom of the container; in positive reactions, they apparently became enmeshed in the virus-antiserum network since they failed to settle; in intermediate reactions, they settled only partially. Positive reactions were obtained with the rosette and type strains of TMV and with 7 mutant derivatives of the type strain in both homologous and heterologous mixtures, but not with Southern bean mosaic virus nor its specific antiserum. The sheep red blood cell test is much easier to carry out than the complement fixation test; it requires less material than the precipitin test.

Correlation of the growth rate of 23 sclerotinia isolates in vitro with rate of infectivity in five hosts at five different temperatures. MORGAN, O. D., JR. Isolations of *Sclerotinia* spp. from red and ladino clover, alfalfa, turnip, lima bean, carrot, daisy, cabbage and delphinium were tested at 40°, 50°, 60°, 70°, and 80° F. to compare rate of growth in vitro with infection on red and ladino clover, alfalfa, carrot and cabbage. Each host was tested separately along with sets of 23 plate cultures at each temperature. In general the most rapidly growing cultures in vitro produced greatest rate of infection at all temperatures. Isolates from lima bean, cabbage, carrot, daisy, delphinium and isolates 7, 8, 9, 13 and 16 from field legumes produced slight infection at 40° and 50° F. with the best temperatures at 60° and 70° F. Optimum growth in vitro was at 70° F. The best temperatures for infection of isolates from turnip and field legume isolates 10, 11, 12, 14, 15 and 17-23 are at 50° and 60° F. with optimum growth in vitro at 60° F. There is a wide variation between isolates both in growth rate in vitro and in amount of infection of the various hosts at the different temperatures. The amount of mycelial growth at the 3 lower temperatures in vitro was directly correlated with amount occurring on the hosts.

Some effects of eight fungicides on growth of Sclerotinia fructicola, Glomerella cingulata, and Penicillium expansum. MORGAN, O. D., JR. Cultures were grown on whole-wheat agar plates containing various concentrations of Cu_2O, $CuSO_4$, $CuCl_2$, Bordeaux mixture, Bioquin-1, Bioquin, Fermate, and Puratized. Two concentrations in duplicate were used in each transfer. A plate to plate mass inoculum method was used; controls were included. Twenty transfers were made over a 13-month period. Following the tests, 10 transfers were made to fungicide-free media to determine whether permanent changes had occurred. All chemicals reduced rate of growth of all fungi exposed. In most instances this characteristic was retained. Permanent loss of spore production occurred in *G. cingulata* on $CuSO_4$ and Bioquin and in *S. fructicola* on Puratized. Permanent reduction of aerial growth occurred in *G. cingulata* on Cu_2O. Permanent color changes occurred in *G. cingulata* on Fermate and Bioquin and in *S. fructicola* on Puratized. Most chemicals produced some color change in *G. cingulata* during the exposure. *P. expansum* produced a copper color while exposed to Cu_2O, $CuSO_4$, $CuCl_2$ and Bordeaux mixture. Heavy production of microspores with loss of macrospore production occurred in *S. fructicola* exposed to $CuCl_2$ and $CuSO_4$. Permanent heavy production of coremia occurred in *P. expansum* exposed to Fermate, Bioquin-1 and Bioquin. Resistance developed in 10 instances.

Chemical constitution of quinones in relation to amylase inhibition and fungitoxicity. OWENS, ROBERT G. The effects of 28 benzenoid and quinonoid compounds on pancreatic and malt amylases and on spores of *Sclerotinia fructicola* and *Alternaria oleracea* were compared. Effects generally are analogous and are correlated with molecular constitution of the compounds. Compounds with benzenoid nuclei are relatively inactive at 10^{-4} M, while quinonoid compounds are active or inactive, depending upon substituents on the ring. Halogenation increases activity; hydroxylation or methylation decreases activity. Inhibition by unsubstituted quinones increases as their oxidation potentials increase, while some chlorinated naphthoquinones are more active than benzoquinones with higher oxidation potentials. Inhibition is thus related to oxidation potential and to the ease of ortho substitution, both of which are affected by resonance effects and the electronegativity of substituents. p-Xyloquinone, 2-methylnaphthoquinone, 2,4-dichlorotoluene, and 3,4-dichlorotoulene are relatively more fungitoxic than is indicated by amylase inhibition. Therefore, their action against spores cannot be explained on the basis of chemical interaction with protein molecules of the amylase type alone, but presumably involves other chemical and physical factors.

Spore uptake of fungitoxicants assayed by amylase inhibition. OWENS, ROBERT G. Solutions of .001 μg./ml. of some fungitoxicants inhibit amylases as much as 10 per cent. Based on inhibition curves a sensitive assay method for esti-

mation of chemisorption by fungus spores was developed. Solutions of mercuric chloride and p-benzoquinone at concentrations which would inhibit amylase 0 to 100 per cent were prepared. Fungus spores were incubated for an hour in the solutions and then removed by centrifugation. Amylase inhibition by the supernatant solutions was then compared to aliquots of the same solutions without spores. From 1.6×10^{-5} and 1.5×10^{-5} M solutions of mercuric chloride 7×10^5 spores of *Sclerotinia fructicola* remove 4.09 and 3.86 μg. of mercury respectively, whereas 1.4×10^5 spores remove 2.03 and .74 μg. from 8.4×10^{-6} and 3.0×10^{-6} M solutions respectively, indicating that uptake is a function of the spore/chemical concentration ratio. On a spore weight basis, 3.86 μg. of mercuric chloride is equivalent to 7000 p.p.m. which can cover about 120 per cent of the spore surface in a monomolecular layer. One million *Alternaria oleracea* spores remove 20.8 μg. of p-benzoquinone from a 2×10^{-4} M solution or about 30,000 p.p.m. of the spore weight. This could occupy 4000 per cent of the spore surface. These data indicate that chemicals are rapidly absorbed or converted to inactive forms.

Fungicidal action of sodium dialkyldithiocarbamates. PALMER, H. C., R. W. GREENLEE, AND M. M. BALDWIN. As part of a program to investigate the mechanism of fungicidal action among chemically related compounds, several sodium dialkyldithiocarbamates were studied. Significant differences in fungicidal properties were found (e.g., dimethyl > diethyl > di-n-propyl). A basic buffer system, used to reduce hydrolysis and oxidation of the dithiocarbamates in dilute solution, increased their over-all fungicidal effects, indicating that these salts are more lethal in substantially pure form than as a mixture of the salts and their decomposition products. The dialkylamines were more fungicidal and showed a greater fumigant effect than a second hydrolysis product, carbon disulfide. A technique was devised to determine the relative sorption of S^{35}-labeled dithiocarbamates, as indicated by the radioactivities of thin samples of treated fungus spores. This technique was used to study the relationship between sorption and fungicidal action of the dithiocarbamate homologues.

Constitution of two plant viruses as suggested by effects of deuteron bombardment on their infectivity and serology.[1] POLLARD, E. C. AND A. E. DIMOND. Loss of infectivity by deuteron bombardment of southern bean and tobacco mosaic viruses follows the relation $\log I/I_0 = -SD$ where I and I_0 are final and initial infectivities measured by local lesions, D is deuteron dosage, and S measures the radiation-sensitive area. Deuterons cause loss of serological combining power by a similar relation where S′ measures target size of the serologically active unit on the virus surface. Loss of infectivity by TMV is independent of ionization density, suggesting the entire infective unit is radiation-sensitive and homogeneous. SBMV loses infectivity rapidly at high ionization densities, the target size being the entire virus particle, and slowly at low ionization densities, the effective target being smaller. At 100 times the deuteron dosage for 37 per cent infectivity the serological combining power of TMV is still 95 per cent and of SBMV is 40 per cent. For TMV the serology curve suggests a single radiation-sensitive unit. For SBMV the curve has two linear segments. The initial rapid fall corresponds to a target of 3.2×10^{-14} cm.^{2} (mol. wt. about 12,000) while the ensuing slow loss corresponds to a target of 1.24×10^{-14} cm.^{2} (m.w. 2000). Therefore SBMV has more complex organization than TMV.

A new light-colored race of Helminthosporium sativum. PON, D. S. In 1949, an exceptionally virulent race of *Helminthosporium sativum* was isolated from a leaf of Kindred barley. On potato-dextrose agar it produces pinkish mycelium with light-colored spores. The conidia resemble those of dark-spored races of *H. sativum* in size, shape, and number of septa. The optimum temperatures for mycelial growth and spore germination are the same as those of the black races. On wheat, barley, rye, and grasses the new race causes root-rot, seedling blight, leaf blotch, and head blight like the typical dark-spored races. The new race also attacks oats and corn heavily as well as barley varieties generally considered resistant to *H. sativum* such as Kindred, Manchuria, and Peatland. Many of the standard varieties of spring wheats also are susceptible. The light-colored race is definitely much more virulent than the common dark-spored races.

An improved method of smut inoculation. POPP, W. AND W. J. CHEREWICK. A practical and effective method of inoculating barley and oats with seedling smuts (*Ustilago hordei* and *U. nigra* of barley, and *U. kolleri* and *U. avenae* of oats) has been developed. It consists of violently agitating seed in a water suspension of spores in a Waring Blendor for about 25 seconds followed by slow drying in seed envelopes at room temperature. The agitation loosens and cracks the hulls, and slow drying permits the spores to germinate and develop mycelium underneath the hull. To prevent seed injury, the cutter knives are replaced with similarly-shaped blunt blades. The suspension is maintained at a constant level in the Blendor by replenishing after each seed lot is treated. The inoculum is prepared by churning smutted heads in distilled water in the Blendor until a suspension equivalent to about 2 gm. of spores per liter is obtained. Due to variations in hull thickness and persistence, varieties of grain require slightly different times for mixing, but this is readily determined by seed examination after agitation. Two persons can inoculate and package up to 120 seed lots per hour. The method therefore offers a reliable means of inoculation particularly suitable for large scale tests.

Production of exotoxin in the genus Verticillium. PORTER, C. L., AND RALPH J. GREEN. Several isolates of *Verticillium albo-atrum* R. & B. were compared as to relative pathogenicity and production of a toxic fraction in synthetic culture medium. The toxic fraction isolated from culture filtrate proved to be a complex polysaccharide which is evidently extremely heterogenous in molecular size. This was demonstrated by the fact that at least 2 fractions can be demonstrated, one of which will not pass through a Seitz filter. These 2 fractions exhibit different loci of activity so far as the plant is concerned. A technique was devised to collect rather large quantities of tracheal fluids from tomato plants previously inoculated with a virulent culture of *Verticillium albo-atrum* R. & B. By excising and rerooting such plants in sterile sand, collection of tracheal fluids could be made with relatively little injury to the plant. These tracheal fluids proved to be toxic to tomato seedlings and attempts were made to isolate and characterize the toxic fraction.

Production of disease-free planting stock of Mentha piperita L. PORTER, C. L., AND EUGENE HIMELICK. Mint wilt has been responsible for a serious reduction in yield of peppermint in Indiana and Michigan. To date, adequate control measures do not exist. Clean planting stock (rooted rhizomes) would contribute materially to control. Peppermint rhizomes which were selected in the spring, and again in the fall, were heat-treated at temperatures lethal to *Verticillium*. An exposure of 47° C. for 55 minutes or 48° C. for 35 minutes gave the greatest percentage of viable disease-free rhizomes. Rhizomes were inoculated with *Verticillium albo-atrum* R. & B. to determine the effectiveness of the heat treatment. When using temperatures lethal to *Verticillium* spores, no trace of the disease was found in rhizomes that were inoculated with the fungus 5 days prior to heat treatment. The results of laboratory and greenhouse investigations indicate that disease-free propagating stock can be produced by this method. Field results are not yet available.

The cause of wilting in tobacco plants affected by black shank. POWERS, HARRY R., JR. Physiological studies of tobacco plants affected by black shank, incited by *Phytophthora parasitica* var. *nicotianae*, indicate that the wilt symp-

[1] Assisted by the Atomic Energy Commission and the Office of Naval Research.

tom develops as the result of a localized disruption of water movement within the area of the stem lesion, rather than the systemic action of toxic substances. Wilted plants recovered turgor when water was introduced into the stem above the lesion, but not when water was supplied below the infection site. An aqueous solution of eosine dye moved through the root system to all parts of healthy plants within 4 hours, but did not pass through the stem lesion of diseased plants. Histological studies of basal stem lesions were made at various stages of pathogenesis. Mycelium was found to be abundant in all tissues of older portions of the lesions. At the upper advancing margin of infection mycelium was found only in the pith, internal phloem and adjacent xylem. Abundant, will developed tyloses found in the tracheids and vessels of diseased stems are indicative of the cessation of water movement in these elements. Stem tissue 3 in. above the lesion was indistinguishable from tissue in stems of healthy plants.

Morphology and pathogenicity of Albugo occidentalis, the incitant of white rust of Spinach. RAABE, R. D., AND GLENN S. POUND. Conidia of *Albugo occidentalis* usually germinate to produce zoospores but rarely germinate directly. In indirect germination, the protoplasm swells and a bulge develops. When the weakened wall ruptures, the contents flow out as a protoplasmic mass in which cleavage planes are sometimes visible. This mass immediately begins oscillating and after a short time, the zospores begin to form and pull away from each other. Zoospores are shaped like inverted dugout canoes and move by 2 flagella, one of which has a terminal knob. After swimming a short time, they round up and germinate to produce a germ tube, which may enter the stoma and initiate infection. Inside the host the fungus produces an intercellular mycelium which is abundant in the large intercellular spaces of the spongy parenchyma; here it masses to give rise to the conidiophores and conidia that make up the sori. Frequently numerous hyphae are found in the vascular areas and in this manner, a more rapid spread through the host is effected. Antheridia arise as terminal swellings and oogonia arise either as terminal or intercalary swellings of hyphae. Fertilization apparently follows zonation in the oogonium although many oospores appear to develop parthenogenetically. All attempts to germinate oospores have failed.

Correlation of seedling blight to stalk rot and nature of seedling resistance to Diplodia zeae. REILLY, J. J. Five monoconidial isolates of *Diplodia zeae* differed in virulence on 5 Minnesota hybrid corn varieties. Virulence was measured by the amount of seedling blight caused when uninjured seeds were inoculated and by the amount of stalk rot that developed by inoculating plants with the toothpick method in the greenhouse and in the field. There was always a significant correlation between the amount of seedling blight and the amount of stalk rot caused by individual races on individual varieties. The corn varieties ranged from very susceptible to very resistant. The differences in susceptibility and resistance to the different races were quantitative, as there were no cases of reversal of reaction when the varieties were inoculated with different races. Resistance was correlated with high concentration of sugars in 3-weeks-old seedling roots, as shown by paper partition chromatography analyses. Resistance also was associated with the ability of resistant seedlings to produce secondary roots.

Tolerance of a black fungus to phenols and quinones. RICH, SAUL, AND JAMES G. HORSFALL. Black-spored *Macrosporium* (*Stemphylium*) *sarcinaeforme* is up to 10,000 times as tolerant of phenols and quinones as hyaline-spored *Sclerotinia fructicola*. *Macrosporium* contains intracellular laccase which probably produces the color by oxidizing phenols to quinones. Therefore, *Macrosporium* should be and is tolerant of quinones. Such phenols as are oxidized by laccase are probably detoxified by conversion to quinones. The following phenols are not oxidizable by laccase, and hence should be, and are toxic to *Macrosporium*: hexylresorcinol, 2,4,6-trichlorophenol, *p*-cyclohexylphenol, *p*-phenylphenol, and *o*-phenylphenol. Laccase oxidizes hydroquinone but 2-cyclohexylhydroquinone is highly toxic to *Macrosporium*. 2-Cyclohexylhydroquinone, however, inhibits its laccase activity. *Sclerotinia* spores, lacking color, probably do not form quinones, do not normally live with them, and consequently are poisoned by them. *Sclerotinia* produces intracellular polyphenol oxidase, but the enzyme must be inhibited *in vivo* because the spores are colorless. Hydrogen donors inhibit polyphenol oxidase. That *Sclerotinia* has an active hydrogen donor system is indicated by its production of massive amounts of H_2S from sulfur. *Macrosporium* spores, which produce little H_2S from sulfur, probably lack an active hydrogen donor system.

High concentrations of virus X in plants doubly infected with potato viruses X and Y. ROSS, A. FRANK, W. F. ROCHOW, AND BENJAMIN M. SIEGEL. The ratio of the amount of virus X (mild strain) in juice from Turkish tobacco plants infected with potato viruses X and Y to that in juice from comparable plants infected with virus X alone varies with the stage of infection and with the sequence with which the plants are inoculated with the 2 viruses. For inoculated leaves and for leaves which expand after systemic infection, this ratio increases with time to about 3:1. With plants inoculated simultaneously with both viruses or with plants inoculated first with virus X, there is approximately 10 times as much virus X in juice from leaves showing acute symptoms as in that from similar leaves of X-infected plants. When inoculation with virus Y precedes that with virus X by an interval sufficient for systemic spread of the former, the ratio of the amount of virus X in doubly-infected leaves with acute symptoms to that in comparable singly infected ones is considerably less than 10:1 but greater than unity. Assays by lesions on *Gomphrena globosa* and by electron microscope particle counts (method of Williams and Backus) were in agreement. In general, symptom severity in systemically infected leaves was correlated with virus X concentration.

Antagonism of several isolates of Rhizoctonia solani by a species of streptomyces. RUSHDI, M. K. During the course of experimental work with cultures of *Rhizoctonia solani* a contaminant, apparently belonging to the genus *Streptomyces*, was found which caused extreme inhibition of this organism. This contaminant was obtained in pure culture and was inoculated in the form of a streak across the center of petri plates containing Czapek's agar medium. Antagonism to 11 isolates of *R. solani* was tested by placing inoculum on either side of the central streak. Strong antagonism of this Actinomycete to 9 of the *Rhizoctonia* isolates was readily apparent. Two isolates were antagonized only slightly.

Variation in Rhizoctonia solani. RUSHDI, M. K. AND W. F. JEFFERS. Fifteen isolates of *Rhizoctonia solani* (*Corticium vagum*) from various hosts and representing several of the southeastern states were grown under a variety of environmental conditions. A type culture of *Corticium vagum* was also included. At 40°F. only one (isolate E from lima bean in Maryland) made appreciable growth. At 50°F. all isolates except one from sugar beet leaves and another from soybean made moderate to good growth. At temperatures of 60, 70, 80, and 90°F. all except the type culture made moderate to good growth. Isolates A (from sugar beet), G (from *Euphorbia pulcherrima*) and P (from soybean) made fair growth at 100°F. while all other isolates made little or no growth. When grown on Czapek's medium containing different amounts of sucrose, some made better growth at low levels than at high levels. Other isolates made good growth at all levels, but in all cases there was no apparent pigment formation when sucrose was omitted. All isolates were grown on 6 standard media to study differences which might be apparent under such conditions. Growth was good on all media, but Czapek's gave best results. Isolate P formed large black sclerotia on several media, but none was produced on yeast-dextrose agar. All other isolates except A formed small, brown, irregular sclerotia on all media except yeast-dextrose agar. Pathogenicity tests indicated that all of the 16 isolates

produce lesions on roots and stems of sugar beet seedlings.

Studies on the entry of larvae of root-knot nematodes into roots of susceptible and resistant plants. SASSER, J. N., AND A. L. TAYLOR. This work is an extension of that of Barrons and Christie on resistance of plants to attack by root-knot nematodes. With all the host species tested, it has been found that in addition to normal entry and development of the root-knot larvae, there are also other reactions depending on the plant species and nematode species involved. These are: (a) failure of the larvae to enter the roots; (b) entry of reduced numbers of larvae with little or no development; (c) entry of large numbers of larvae with varying degrees of development ranging from none at all to a few of the individuals reaching maturity. Thus resistance is not of the same nature in all plants. The nature of resistance cannot yet be explained, but practical use can be made of these ovservations. Plants in which the larvae enter freely, but do not develop would act as trap crops and so would be more effective in a nematode reducing rotation than would plants not entered at all.

Short-season adaptations in the rust fungi. SAVILE, D. B. O. The northern limit for heteroecious, self-sterile, long-cycle species of *Puccinia* and*Uromyces* is approximately tree line. Self-fertility and the short-cycle habit enable many species to persist in the arctic on suitable hosts. In *Chrysomyxa*, overwintering of dikaryotic mycelium in evergreen leaves permits simultaneous development of uredinia and telia, and also makes the rust independent of the aecial host. *Hyalopsora aspidiotus* overwinters both by amphispores and by mycelia in the aecial host, thereby producing aecia, uredinia and telia nearly simultaneously. *Melampsora epitea* overwinters as a systemic-perennial monokaryon in *Saxifraga* and as a dikaryon in *Salix* buds, as well as by telia, thus being able to survive even when the sexual part of the life cycle cannot be completed.

Distribution of Fusarium resistance in the tomato plant. SCHEFFER, R. P. AND J. C. WALKER. Several workers have offered evidence to show that resistance to *Fusarium* wilt in tomato is localized in the roots. To test this hypothesis, a cutting inoculation technique was devised. Cuttings were taken from young (5-7 weeks old) vigorously growing plants of highly resistant (single gene), moderately resistant (multiple gene), and susceptible varieties. The cut ends were immersed in dilute suspensions of microconidia and kept for several hours under conditions favoring relatively high transpiration. Next the basal stem ends which contacted the conidial suspension were cut off, the cuttings were transferred to a fresh nutrient solution and then placed for several days in a lighted moist chamber at 22°-24°C. After roots appeared the cuttings were potted in sand and placed under conditions favoring disease. Ten to 15 days after inoculation, wilt and vascular discoloration symptoms appeared in all varieties. At this time the causal fungus could be isolated from the wilted petioles but not from the roots. Resistant varieties, however, soon recovered and grew normally, while in susceptible varieties wilt became progressively more severe. These results are considered as evidence that resistance to *Fusarium* wilt is not localized in the roots but occurs throughout the plant.

Hydrolysis of pectin and cellulose by isolates of Colletotrichum phomoides, C. lagenarium, and Glomerella cingulata. SCHMITTHENNER, A. F. As part of an investigation of the factors involved in the spread of *Colletotrichum phomoides, C. lagenarium,* and *Glomerella cingulata* through apple and tomato fruits, a study of the hydrolysis of cell wall materials by an isolate of each of these fungi has been initiated. The cellulase activity and pectinase activity of the isolates were compared by measuring the day to day increase in reducing power of glycerol-nitrate cultures of the isolates containing 0.5 per cent powdered filter paper and commercial pectin, respectively. Reducing power was determined through the hypoiodite method used by Jansen and MacDonnell (1945). Hydrolysis of both pectin and cellulose was most rapid in the *G. cingulata* cultures. Hydrolysis of pectin was complete in both the *G. cingulata* and *C. phomoides* cultures, and not complete in the *C. lagenarium* cultures. No cellulase activity was demonstrated in the *C. lagenarium* cultures.

The Influence of pH and zinc on vesicle formation in Puccinia coronata Avenae Corda. SHARP, E. L. AND F. G. SMITH. The formation of structures corresponding to appressoria, substomatal vesicles, and infection hyphae of *P. coronata* was induced on 3 per cent gelatin by the adjustment of pH and the addition of Zn. As high as 40 per cent of the germinating uredospores formed these structures under optimum conditions (pH 6.2 to 6.6 and 40 to 63 p.p.m. of Zn). Zn and pH levels also influenced branching adjacent to the spore and the optimum conditions were similar to those for vesicle formation except for a somewhat wider pH range. Uredospores germinating on water or water-agar at various pH levels and Zn concentrations have not shown vesicle formation although branching was prevalent. Fe, Cu, Mo, Mn, Ca, and Mg will not substitute for Zn.

A quantitative electrophoretic study of competition between two strains of tobacco mosaic virus. III. The effect of inoculum strain ratio and the method of inoculation on the strain ratio in the systemically infected leaves. SIEGEL, ALBERT, MORRIS COHEN, AND SAM G. WILDMAN. When equal amounts of a mild strain and the common strain of *TMV* are applied to a single fully-expanded leaf of a tobacco plant, the rest of the plant may become infected with only 1 of these strains, although in the inoculated leaf the amount of the common strain only slightly exceeds the mild strain 25 days after inoculation. When various mixtures of these 2 strains are applied to several fully-expanded leaves of a tobacco plant, the rest of the plant usually becomes infected with both strains and the plants eventually show the symptoms of the common strain even when the inoculation mixture contains only 1 per cent of the common strain to 99 per cent of the mild strain. With inoculation mixtures of common and mild strains containing 1 or 50 per cent of the mild strain, the amounts of the mild strain present in sets of systemically-infected leaves at successively higher levels in the plant were 10, 40, and 10 per cent. When 99 per cent of the inoculation mixture was the mild strain, there was a continuous drop in the per cent of the mild strain at successively higher levels in the plant.

Effects of various temperatures upon symptomatology of wheat yellow streak-mosaic virus. SILL, W. H. JR. Wheat plants, vars. Lee, Rival, Marquis, Westar, and Michigan Amber, were grown under winter light conditions, 6 per 6-inch pot at 25° C. At the 2-leaf stage they were divided into 5 groups and were kept for 1 week prior to inoculation at air temperatures of 28°, 24°, 20°, 16°, and at 16° with an 8° soil temperature. The incubation period lengthened with decreasing temperatures, the average of 3 trials being 5 days at 28°, 7 at 24°, 9 at 20°, 15 at 16°, and 23 at 16° with an 8° soil temperature. Most severe symptoms appeared first at 28° but shifted to 24°, then to 20°, and by 2 months to 16°. Vars. Marquis, Westar, and Michigan Amber finally showed slight symptoms at 16° with an 8° soil temperature, but Lee and Rival did not, although infective virus was obtained from the plants. These plants when shifted to 24° developed marked symptoms. Stunting was slight to absent at the 2 lowest temperatures (av. 3 per cent). In contrast, the average for all varieties was 16 per cent at 20°, 20 per cent at 24°, and 18 per cent at 28°.

Physiologic specialization in Puccinia coronata Corda var. avenae F. & L. SIMONS, MARR D. AND H. C. MURPHY. A total of 18 differential varieties of oats were used to identify collections of crown rust in 1950. These varieties were grouped as follows: (a) the standard 13 differential varieties, (b) a new set containing Anthony, Victoria, Appler, and Bond, of the standard set plus Landhafer, Santa Fe, Ukraine, Trispernia, and Bondvic, and (c) the combined set of 18 varieties. In 1951 Saia was added to the new and combined sets. In 1950, 14 races were differentiated using the old set. Two new races, numbered 101 and

102, were described. Using the new set 27 races were identified, while the combined set differentiated 43 races. In 1951, 16 races were identified using the old set. Five new races, numbered 103, 104, 105, 106, and 107 were described. Using the new set 27 races were identified, while the combined set differentiated 44 races. Relative efficiency of the sets was calculated on 3 bases: (a) total number of races identified, (b) number of races in relation to the number of varieties comprising each set, and (c) number of races that theoretically could be identified by each set. It was recommended that the new set of 10 varieties used in 1951, be used henceforth to identify races of crown rust.

Effect of tetramethylthiuram disulfide on anaerobic breakdown of glucose by fungi. SISLER, H. D., AND C. E. COX. A study was made of the effect of tetramethylthiuram disulfide (TMTD) on the steps involved in the anaerobic breakdown of glucose in fungus metabolism. Manometric techniques have shown that TMTD inhibits anaerobic fermentation (CO_2) of glucose by living cells of several fungi and of glucose and fructose-1,6-diphosphate by preparations of dried brewers yeast cells. Approximately equal inhibition of fermentation by yeast preparations of equivalent amounts of these 2 substrates by the same concentration of fungicide indicates that it exerts no effect upon steps intermediate between these 2 compounds. However, TMTD does not affect fermentation of 3-phosphoglyceric acid and pyruvic acid by dried yeast preparations. Cysteine and glutathione completely offset the effect of TMTD on fermentation of fructose-1,6-diphosphate by the dried yeast preparations. The effect of the fungicide on utilization of glucose by living yeast cells likewise is almost completely eliminated by cysteine and glutathione. Colorometric techniques have shown that TMTD prevents conversion of fructose-1,6-diphosphate to 3-phosphoglyceraldehyde and dihydroxyacetone phosphate. The effect of TMTD on this reaction is prevented by glutathione. The studies indicate that TMTD affects fungus utilization of glucose by preventing conversion of fructose-1,6-diphosphate to the 3-carbon intermediates, dihydroxyacetone phosphate and 3-phosphoglyceraldehyde.

Studies on host-parasite relations of bean varieties resistant and susceptible to Pseudomonas phaseolicola and toxin production by the parasite. SKOOG, H. A. Population trends of bacteria within the leaf tissue in resistant Mexican Red and susceptible Red Kidney varieties were determined following uniform inoculation with *Pseudomonas phaseolicola.* Uniform inoculation was accomplished by water-soaking with a standard preparation of inoculum. Increases in viable bacteria were very similar in the 2 varieties the first 3 days after inoculation. A further increase was obtained in the susceptible variety accompanied by necrosis and collapse of leaf tissue about the sixth day. A lower population peak in the resistant variety was reached on the fifth day followed by a decrease. Toxin production by *Pseudomonas phaseolicola* was demonstrated by growing pure cultures in glucose broth from 10-14 days at temperatures of 10° and 15° C. Little or no toxin was obtained at growth temperatures of 20° C. or higher. The toxin was thermostable and readily detected by the needle puncture method on young bean leaves. It has been reported that typical halo symptoms develop on inoculated beans chiefly at temperatures of 20° C. and below. This temperature range corresponds closely with that obtained for the production of toxin in pure culture.

Dodder transmission of a virus from X-diseased peach and sand cherry. SLACK, DERALD A. In greenhouse experiments, stems of dodder, *Cuscuta subinclusa* Dur. & Hilg., were trained from X-diseased peaches and sand cherries to herbaceous plants. After haustorial connections were established, the herbaceous plants were darkened to facilitate virus transfer. A virus was transmitted from diseased trees to periwinkle, carrot, parsley, jimson weed, and strawberry (*Fragaria vesca* L., East Malling clone). The virus was transmitted from diseased herbaceous plants to healthy ones by dodder and grafting. Attempts to transmit this virus to other plant species are in progress. Disease development in periwinkle, carrot, and parsley follows a similar pattern to that reported by Kunkel (Phytopath. 34: 1006.) Epinasty and chlorosis of the leaves occurred on affected jimson weed plants; developing leaves were strap-shaped and chlorotic. Leaves of diseased strawberry plants became markedly rugose and stunted; chlorotic and necrotic streaks occurred on distorted leaves. Diseased plants of all species developed numerous secondary shoots. Control herbaceous plants connected to healthy trees by dodder did not express symptoms of disease. Experiments aimed at identifying the virus transmitted to the herbaceous plants are in progress.

Streptomycin sulfate for control of bacterial soft rot of packaged spinach. SMITH, WILSON L., JR. Spinach was sprayed with streptomycin sulfate (0.1 per cent) plus Tween 20 (0.5 per cent) 5 days or 1 day before harvest. At harvest it was washed in water containing soft rot bacteria, packaged in pliofilm sacks, and held at 70° F. No decay was evident after 1 day, decay was rarely evident after 2 days, but many sacks were nonsalable in 3 days. They became mushy masses in 4 to 5 days. Spinach field—sprayed at the same time with just Tween 20 (0.5 per cent) and then washed in a bacterial suspension showed decay in 1 day, was nonsalable in 2 days, and was a mushy mass in 3 days. Dipping nonsprayed freshly-harvested spinach in water containing soft rot bacteria and streptomycin sulfate (0.1 per cent) plus Tween 20 (0.5 per cent) gave better decay control than field spraying. Instant, 5-, and 10-minute dips were equally good. Spinach dipped in Tween 20 (0.5 per cent) plus bacteria showed slightly more decay than the field-sprayed. Sprayed spinach stored 2 days at 32° decayed faster at 70° than freshly harvested sprayed spinach. Non-sprayed spinach stored 2 days at 32° and then dipped also showed faster decay at 70° than the freshly harvested dipped.

Contribution to the life history and control of snow molds of winter wheat in Washington. SPRAGUE, RODERICK. Most of the basidiospores from fruiting bodies of *Typhula* spp. that are growing on wheat stubble in the fall fail to travel very far but some carry infection to newly seeded wheat. Spores discharged in late October, 1951, caused mold in wheat grown in previously mold-free soil placed in board-walled replicated plots in diseased fields. Deep-spading also failed to eliminate the mold. New fields just broken from the sagebrush but surrounded by dead *Typhula*-carrying *Bromus tectorum* were heavily molded. New pastures and also lawns seeded on mold-free subsoil in the Columbia River Valley several miles from known infection were injured by *T. itoana* following the prolonged snows of 1951-52. Practical chemical control of snow molds (*T. itoana, T. idahoensis* and *Fusarium nivale*) was obtained with Ceresan-M applied to the young wheat in early November, 1951 as a damp dust at the rate of 4 lbs. 11 oz. per acre. In areas where it is necessary to reseed with spring wheat because of snow mold this promising treatment may prove commercially feasible.

Factors affecting local control of white pine blister rust. STEWART, DONALD M. To eliminate blister rust cankers caused by *Cronartium ribicola* Fischer from young planted eastern white pine, *Pinus strobus* L., infected trees were pruned in 3 different ways: (a) where canker margin was 6 in. from trunk entire branches were cut off; (b) where canker was nearer trunk, branches were removed and bark around branch stub excised; (c) where canker was on upper main stem, the top of tree was lopped-off below canker margin. Reexamination 3 years after pruning indicated 100 per cent of trees in first group were canker free, 69 to 100 per cent in the second group, and 95 per cent in the third group were free of blister rust cankers. Canker elimination was attempted on natural forest trees by (a) removal of diseased limbs only and (b) removal of all lower branches and those with cankers in the upper 5 whorls. Examination after 6 years showed the second

method more successful. Eradication of *Ribes* was studied on 2 burned-over areas, 1 initially burned 26 years and the other 2 years previous to planting to eastern white pine. On the old burn where the *Ribes* population had become static, 1 eradication job gave effective control. On the recent burn abundant *Ribes* regrowth had occurred after the fire and required 3 separate workings before the *Ribes* population had become reduced and stabilized.

Chemotherapeutic control of Rhizoctonia on greenhouse stock. STODDARD, E. M. Root injury by *Rhizoctonia* sp. causes either stunting and death of young stock plants, *Matthiola incana*, or severe wilting of mature plants. This injury by *Rhizoctonia* can be controlled by soil applications of chemicals. Chemotherapeutic action is indicated. Three replicates of 10 plants each planted in infested soil received 5 soil applications of 8-quinolinol sulfate at a concentration of 1-4000 in 14 days. All plants grew normally with healthy root development. Three similar lots of plants in the same lot of soil without treatment were all killed or severely stunted by root infection of *Rhizoctonia*. Four treatments in 10 randomized blocks comprised a second trial as follows: (a) infested soil; (b) infested soil plus 10 successive treatments of 8-quinolinol sulfate 1-4000; (c) sand plus 10 treatments of 8-quinolinol sulfate, plants removed from sand after treatments, roots thoroughly washed and replanted in infested soil without further treatments; (d) sand, no treatment. Only plants in treatment 1 showed wilting of mature plants. Treatment 3 indicated chemotherapeutic action of 8-quinolinol sulfate. One greenhouse trial of this material on a commercial planting of stock was entirely successful. Two other similar trials gave less control but were commercially profitable.

Changes in the soil flora of banana lands flood fallowed for the eradication of Fusarium oxysporum cubense. STOVER, R. H., N. C. THORNTON AND V. C. DUNLAP. Flood fallowing was introduced in 1940 by the United Fruit Company to eradicate *Fusarium oxysporum cubense* from infested land. Clay loam soils are submerged up to 6 mo. under 2 to 4 feet of water. Wind and inflow circulate the water so that little oxygen stratification occurs. The greatest decrease in the fungus flora occurs around the thirty-fifth day of submergence, shortly after a great rise in the bacterial flora of the surface mud; after 120 days, most of the indigenous fungus flora is eradicated. However, a small fungus population is found at all times on the submerged soil, derived largely from inflow-water and silt. Inflow-water contains an average of 30 to 150 spores per ml. Indigenous *Fusarium* spp. are seldom found in clay loam soils inundated for 40 days. However, *Fusarium* spp., and occasionally *F. oxysporum cubense*, derived from the migratory water flora are found at all stages of flooding. After 30 to 60 days of submergence, there are 8 to 10 times as many fungi (mostly *Penicillum* spp.) surviving in sandy loam soils as in clay loam soils. After flooding, *Penicillum* and *Trichoderma* spp. are the first to invade the soil. Flooded soils are more favorable to growth of *F. oxysporum cubense* than non-flooded soils; growth is optimum in all soils at a moisture content of 25 per cent of the field capacity.

Microbiological content of grain dust. SWAEBLY, MARY ANN, AND CLYDE M. CHRISTENSEN. The numbers and kinds of microorganisms in grain dust were determined by microscopic examination and by culturing diluted suspensions of the dust in different agar media. All samples of dust so examined were found to contain very large numbers of a great variety of fungi and bacteria. Species of *Aspergillus* and *Penicillium* comprised a large proportion of the culturable fungi in most of the samples. *Geotrichum* was abundant in some samples. Spores of various rusts and smuts were observed in most samples, as well as spores, mycelium, and occasional sporophores of other fungi. In general, the count of bacteria far exceeded the count of culturable fungi. Other organic materials identified were: fragments of seed coats, endosperm, hairs and other plant parts; pollen grains; body fragments and droppings of various insects: living and dead mites, grain beetles, and grain moths; and animal hairs.

Nutritive value of cornstalk juice inversely related to stalk rot resistance. TAYLOR, GORDON S. To determine if resistance to Diplodia stalk rot in corn is derived from nutritional properties of the stalk juice. A test was made by growing *Diplodia zeae* in 4 replications on agar media containing juice from internodes of inbred corn lines B6 (resistant), L289 (intermediate), W22 (susceptible), and M14 (susceptible). Mean diameters of colonies were for the 4 corn lines respectively 7.9, 6.4, 7.3 and 5.9 cm. Mean colony diameter on plain agar check was 5.5 cm. Nutritive value of stalk juice was inversely related to stalk rot resistance. A similar relation was found with juice from nodal and internodal tissue of B6 and of L289. Nodal tissue retards rot development more than does internodal tissue. Nodal juice supported more growth of *Diplodia zeae* than did internodal juice. Relative resistance to Diplodia stalk rot in the corn lines examined did not result from a direct nutritional effect on *Diplodia zeae*.

Liberation of spores from natural reservoirs facilitates overland spread of oak wilt. TRUE, R. P., J. M. STALEY, J. G. LEACH, H. L. BARNETT AND C. K. DORSEY. As oak wilt trees die, *Chalara quercina* grows from the wood into the bark. Mycelial mats form in small shrinkage-cavities in the cambium and cork cambium regions. Conspicuous fungus cushions, composed in part of pseudoparenchyma and characterized by daedaloid contact surfaces, develop at the cambium. As these pressure cushions grow and swell, they thrust the bark from the wood and rupture it. Within the cavity between bark and wood the fungus sporulates abundantly and gives off a characteristic odor that is apparently attractive to insects. Insects removed from mats of B compatibility and placed upon mats of A compatibility induced perithecia formation within 3 days. Insects can thus spermatize mycelial mats and are also potential vectors. Although endoconidia are formed during all stages of mat development, perithecia have only been found on mats beneath ruptured bark. Both endoconidia and ascospores may be carried overland from these open reservoirs of inoculum by nitidulids, scolytids, and other insects commonly found associated with the sporulating mycelium. These facts indicate that control measures, to be effective in preventing overland spread, must be taken before pressure cushions break the bark.

Fungi important in storage of barley. TUITE, J. AND C. M. CHRISTENSEN. Barley seed was found to contain a variety of fungi existing as dormant spores on the outside of the seed, as mycelium, sclerotia, and occasionally as spores within the hulls and to a lesser extent within the pericarp. Yeasts were present both within the hulls and pericarp. Many of the fungi such as *Alternaria*, *Cladosporium*, *Fusarium*, *Geotrichum*, and yeasts, which were often present in large numbers, either decreased or remained constant when seed was stored in aerated containers at moisture levels from 12.5 to 17 per cent. *Aspergillus glaucus*, *A. versicolor*, and *Penicillium sp.* increased at moisture contents over 14 per cent; their increase was associated with decrease in seed viability. *A. glaucus* was most active in storage at the distal and basal portions of the seed. It was also isolated from a large percentage of embryos of seed whose viability had decreased.

The control of loose smut of barley (Ustilago nuda) by Spergon-SL. TYNER, L. E. Samples of various varieties of barley seed carrying a heavy infection of loose smut were soaked in tap water for 6, 8, or 10 hours at room temperature. The seed was then drained and 0.2 per cent Spergon-SL suspension added. The samples remained in this suspension for periods of 40, 44, or 48 hours. Loose smut was controlled in all varieties to essentially the same degree as may be expected from the standard hot water treatment, and was complete in most cases. Equally satisfactory control was effected by soaking the seed in water at room temperature for 64 hr.; however, disagreeable decomposition was evident.

Strain of tomato ringspot virus from American elm.

VARNEY, E. H., AND J. DUAIN MOORE. A virus inciting rings on tobacco was transmitted mechanically from mosaic infected elms. Its physical properties were: dilution, few rings at 1-1000; aging in vitro, 4-5 days; thermal inactivation, 60° C. Cross-protection studies showed one-way protection with Price's tomato ringspot virus (TmRSV). The elm virus (EV) did not protect against TmRSV, but TmRSV did protect against EV. There was no protection with a strain of tobacco ringspot virus. EV plus potato Y virus gave no synergistic effect. On tomato, EV did not incite symptoms but could be recovered. It also differed from TmRSV on 2 other mutual hosts in that no symptoms were evident on *Nicotiana glutinosa* and usually no local lesions were formed on cowpea. Necrotic lesions developed on elm leaves rubbed with juice from cowpea or tobacco infected with either EV or with TmRSV. These lesions enlarged with the necrosis sometimes spreading into and along the stem. An elm seedling mechanically inoculated with juice from infected elm showed mottle on all leaves the following year. On the basis of similarities of physical properties, symptoms, and host range and the cross-protection tests, it is suggested that the virus from elm is related to tomato ringspot virus.

The significance of raspberry yellow rust control. VAUGHAN, EDWARD K. Spray tests over a period of 5 years have shown that the incidence of raspberry yellow rust, *Phragmidium rubi-idaei*, on the foliage of the fruiting canes is markedly reduced by a single application of fungicide at the green tip stage. Lime sulphur (4 gal./100 gal.), Krenite (0.5 gal./100 gal.), Ferbam (3 lb./100 gal.), and Ziram (3 lb./100 gal.) have given comparable control with no evidence of phytotoxicity. Subsequent application, however, has not resulted in further reduction of the disease. Although severe infections unquestionably reduce both quantity and quality of the fruit, there has as yet been no evidence of a correlation between the observed reduction in yellow rust and the recorded yields of berries. Reducing the number of individual infections apparently is of benefit to the grower only when uncontrolled infections are so heavy as to cause excessive loss of moisture during the harvest season.

Studies on control of white mold of beans. VAUGHAN, EDWARD K., AND B. F. DANA. White mold, caused by *Sclerotinia sclerotiorum*, causes severe losses in fields of the blue lake type of bean in the Pacific Northwest in certain seasons, and is always a threat to that crop. Significant reductions in number of centers of aerial infection have been obtained by the use of sprays and dusts. Over a period of 4 years Ziram (3 lbs./100 gal., and dust containing 7 per cent active ingredient) and Cop-O-Zink (6 lbs./100 gal., and dust containing 6 per cent metallic copper) have consistently given the best controls. Sprays have given consistently better controls than dusts (in 1951 a maximum reduction of 78.7 per cent for sprays; 67.8 per cent for dusts). Applications were made at 7-day intervals from mid-July until the end of the harvest season so that there was a maximum of fungicidal residue on the pods at the time of harvest. Canning tests have indicated, however, that the residues from both materials are readily removed by thorough washing and that objectional residues, discoloration of the cans or the beans, or undesirable flavors can be easily avoided. Improved aeration and more rapid drying of the plants each morning as a result of wider row spacing has also resulted in significant reduction in infection.

Prediction of field performance of fungicides. WAGGONER, PAUL E., SAUL RICH, AND J. G. HORSFALL. Field performance of a fungicide is governed by fungitoxicity, tenacity and stability. Laboratory measurements of each of these factors are related to field performance, other factors being equal. For more precise prediction of field performance of fungicides, however, it is necessary to combine these measurements into a single expression relating them to primary infection on plants. This can be done by assuming a linear relation, (a) between the slopes of the dosage mortality curves as obtained in the laboratory and field, and (b) between probit spore-kill scale and Horsfall-Barratt disease scale. Then the amount of primary infection on plants = A [(slope of dosage-mortality curve obtained in laboratory) (ED 50)] + B [(slope of dosage-mortality curve obtained in laboratory) (log of initial residue in field)] + C (slope of wash-off curve) + D (slope of decomposition curve) + E. This function, except for values of parameters A, B, C, D, and E, applies also to secondary infection by a pathogen which fruits during periods when spray residues are being washed. Given laboratory and field information, the parameters can be estimated by regression analysis. Usefulness of the functions can then be established because the parameters have physical meaning.

Relation of host nutrition to the concentration of turnip virus 1 in Nicotiana glutinosa L. and N. multivalvis L. WEATHERS, LEWIS G. AND GLENN S. POUND. The relative concentrations of turnip virus 1 in expressed extracts of plants of *Nicotiana glutinosa L.* and *N. multivalvis* L. growing under different levels of nitrogen, phosphorus, potassium, and balanced Hoagland's solution in quartz sand were determined by local lesion assays to tobacco. In general, the effects of ntirogen, potassium and concentration of balanced solutions on virus concentration closely paralleled the effects on host growth. The effect of increasing amounts of nitrogen on virus concentration closely paralleled the increase in plant growth and was not dependent on the amount of nitrogen supplied to the host. At nitrogen levels high enough to effect stunting corresponding reductions in virus concentrations occurred. Varying the potassium had little effect on plant growth and likewise little effect on virus activity. Increasing the potassium increased virus concentration providing it also increased plant growth. Increasing concentrations of Hoagland's balanced solutions resulted in increased virus concentration only if growth was also increased. Increasing amounts of phosphorus gave corresponding increases in virus concentration even though the highest level of phosphorus was sufficient to inhibit plant growth.

Breakdown of various organic sulfur fungicides to yield carbon disulfide and its significance in their toxic action. WEED, RICHARD M., S. E. A. MCCALLAN, AND LAWRENCE P. MILLER. Various dithiocarbamates, such as ferbam and nabam, and some fungicidally active xanthates, break down slowly when suspended in aqueous media, to yield carbon disulfide. Ferbam, labeled with S^{35} will give off radioactive carbon disulfide when placed on plants. This can penetrate nearby plants and radioactive sulfur will be present in the organic sulfur compounds and sulfate within these plants. The breakdown of these fungicides to yield carbon disulfide is hastened tenfold or more by the addition of yeast cells or fungus spores. Comparative studies of the toxicity of the vapor given off by nabam and that of comparable quantities of carbon disulfide which could be released indicate that the volatile toxicant is not carbon disulfide. The volatile toxicant from nabam is more active fungistastically and much more active fungicidally than carbon disulfide. The decomposition of these fungicides to yield carbon disulfide is greatly affected by small increases in the hydrogen ion concentration, and the acceleration produced by contact with yeast cells and fungus spores can probably be explained on this basis. These investigations were supported by the Atomic Energy Commission.

Studies of the masking of little cherry disease symptoms. WELSH, MAURICE F. In Kootenay cherry plantings that have been considered 100 per cent infected with the little cherry disease for 6 years or more, 3 Lambert trees have been identified in which no symptoms are detectable. It has been demonstrated, both by inoculation of the parent trees and by transfer of buds from the parent trees to healthy trees, that 2 of the trees are symptomless carriers, and the third a disease escape. When buds from the 2 symptomless infected trees have been applied to other diseased Lambert trees, the growth from these buds has borne fruits with typical little cherry symptoms. Two other sweet cherry

varieties, demonstrated to be carriers of little cherry virus, have produced normal fruits when applied to diseased Lambert trees in the same manner.

Woody plant hosts of the tobacco ringspot virus. WILKINSON, R. E. Systemic infection with the tobacco ringspot virus (*Annulus tabaci*) has been obtained in several woody plant species by mechanical inoculation of seedlings. Some species are easily infected, while others are infected only with difficulty. Preinoculation shading and postinoculation high temperatures appear to favor infection of some species. Following their first period of winter dormancy, the virus was recovered from new growth on these trees by mechanical inoculation to tobacco. Symptoms vary in the different species and with different strains of the virus. In general, the symptoms produced by the type strain are: mosaic and stunting on *Prunus avium* and *Ulmus americana*, mosaic and narrowing of the leaves on *Acer negundo* and *Fraxinus pennsylvanica*, ringspots and line patterns on *Sambucus canadensis*, and mild mosaic on *Hibiscus syriacus* and *Acer ginnala*. *P. avium* and *A. negundo* tend to recover from symptoms, and eventually to produce healthy leaves. The other species appear to remain systemically infected once the virus has invaded the growing points. The tobacco ringspot virus was isolated from the leaves of naturally infected plants of *S. canadensis* which exhibited ringspots and line patterns. These symptoms occur frequently in nature on this species.

Local lesion hosts for the lettuce mosaic virus. WILKINSON, R. E., AND URSULA HIRSCH. *Gomphrena globosa* and *Chenopodium urbicum* react by the production of local lesions 10-14 days following carborundum inoculation with the lettuce mosaic virus (*Marmor lactucae*). These test plants were found to be suitable for quantitative virus assay when they first reached the flowering stage. *G. globosa* is preferred because its leaves occur in pairs and are of more desirable size and shape, and the lesions produced are more distinct than are those of *C. urbicum*. Infection generally is local, but in *G. globosa* it may eventually become systemic under prolonged high temperatures. *G. globosa* was used for assay of virus in juice from inoculated lettuce plants. It was found that, at 33 days after inoculation, the first 3 leaves to show systemic symptoms have a higher virus titer than do the 6th, 7th, and 8th leaves to show symptoms. These first 3 leaves have a higher virus titer 17 days after inoculation than they do 33 days after inoculation. The greatest number of lesions was obtained with inoculum prepared when the infected lettuce leaves were ground with enough 10 per cent sodium bisulfite to give about 2 per cent of the chemical in the expressed juice.

The effect of conidial matrix, hydrogen ion concentration, and thiamine on spore germination and pathogenicity of Colletotrichum lagenarium. WILLIAMS, LANSING, AND PATRICIA ALLISON. The initial optimum pH for germination of conidia of an isolate of *Colletotrichum lagenarium* in 2 per cent glucose was 3.5, with a secondary peak at 11.5. No germination occurred at pH 2.0 or pH 12.0. Usually a higher percentage of germination occurred in suspensions of unwashed conidia (containing 50,000 conidia per ml.) than in corresponding suspensions of washed conidia. A greater number of lesions developed on cucumber leaves when unwashed conidia were used as inoculum than when washed conidia were used. Thiamine hydrochloride, at concentrations of 6-85 p.p.m., in distilled water used as the germination medium, at pH 3.5, had an inhibitory effect on the germination of unwashed conidia, but in concentrations above 85 p.p.m. germination was similar to or greater than that of unwashed conidia in media to which no thiamine had been added. The percentage of germination of washed conidia was greater than that in water controls in all concentrations of thiamine tested (6-1000 p.p.m.). Fewer lesions developed on cucumber leaves inoculated with suspensions of unwashed conidia containing 63-2000 p.p.m. of thiamine hydrochloride than on leaves inoculated with corresponding suspensions with no added thiamine. The same concentrations with washed conidia resulted in greater numbers of lesions.

Preliminary study on the relation between carbohydrate-nitrogen supply and disease production by Hendersonula toruloidea. WILSON, E. E. Young walnut trees grown in a nitrogen deficient soil or girdled at the base by removal of a ring of bark, upon inoculation with *Hendersonula toruloidea* developed more extensive symptoms than trees not so treated. Since such treatments tend to increase the tree's content of carbohydrate in relation to its content of nitrogen, studies were made on the carbohydrate-nitrogen requirements of the fungus. The fungus grew and sporulated rapidly when supplied dextrose, levulose, sucrose, raffinose, and maltose; it grew less rapidly when supplied starch and maltose and poorly when supplied xylose. Asparagine and arginine promoted better growth and sporulation than either ammonium or nitrate nitrogen compounds. Other nitrogen compounds utilized to some extent were cystine, tyrosine, edestine, and urea. Increasing carbohydrate from a 0.5 to a 4 per cent level and nitrogen from a 0.04 to a 0.07 level resulted in increased growth of the fungus. Though growth neither increased nor decreased when the carbohydrate level was raised above 4 per cent, it diminished moderately but progressively when the nitrogen level was raised above 0.07 per cent.

Fungicidal action due to selective toxicity and selective absorption. YARWOOD, C. E., AND LOUIS JACOBSON. Healthy leaves and leaves infected with *Erysiphe polygoni, Uromyces phaseoli, Collectotrichum lindemuthianum, Pyrenophora teres, Ramularia Primulae*, and tobacco mosaic virus as well as spores of *Erysiphe polygoni* and *Uromyces phaseoli* were exposed to H_2S^{35}, $H_3P^{32}O_4$, C^{14}-sucrose, and $Cs^{137}Cl$. The conditions of the experiments were such that the radioactivity *per se* caused no detectable physiological changes. Absorption was assayed by a Geiger counter and by radioautographs. In most cases absorption occurred in greater amounts in diseased than in healthy tissues. Since H_2S is regarded as toxic, H_3PO_4 and sucrose as nutritive, and CsCl as inert, there was apparently no relation between selective absorption and toxic or nutritive value. *E. polygoni* conidia absorbed less S per unit dry weight than did healthy host bean tissue, and *U. phaseoli* uredospores absorbed more than host bean tissue. Yet S was lethal to both fungi without significant injury to the host. The selective fungicidal action of S to *E. polygoni* is therefore regarded as exclusively due to its selective toxicity, while the selective fungicidal action to *U. phaseoli* is due to selective absorption with or without selective toxicity. All cases of fungicidal action against living fungi on living hosts are regarded as due to selective toxicity and/or selective absorption.

Rapid serological detection of virus in Cattleya orchids. ZAITLIN, MILTON, A. M. SCHECHTMAN, J. G. BALD, AND S. G. WILDMAN. Virus has been extracted from *Cattleya* orchid leaves and further purified by differential centrifugation to a high state of electrophoretic homogeneity. The purified virus is used as an antigen to induce antibody formation in a rabbit. When serum containing orchid virus antibodies is layered with a crude extract from a small portion of a virus diseased *Cattleya* leaf, a precipitin reaction occurs. A similar preparation from a healthy leaf induces no such reaction. This simple test has been applied to 98 individual orchid leaf specimens selected by an unbiased observer who supplied no information as to whether the specimens were considered virus infected or not. A later comparison of the results of the serological test with the symptom diagnosis show a large measure of agreement. The precipitin method is also capable of detecting virus in symptomless portions of diseased plants, and appears capable of detecting the presence of virus in symptomless carriers. Electrophoresis of the purified virus reveals the presence of 2 components, and may represent resolution of the complex of 2 viruses seen with the electron microscope.

The role of nursery stock in the dissemination of soil pathogens. ZENTMYER, GEORGE A., KENNETH F. BAKER, AND W. A. THORN. Dissemination of plant pathogens is an important phase of the cycle of disease development and is

frequently neglected in considering control measures. Movement of soil pathogens by means of infected nursery stock or nursery stock growing in infested soil can be important. Investigations with the root pathogen *Phytophthora cinnamomi* Rands, indicate that movement of nursery material may be the primary means by which this pathogen has become so widely distributed. This fungus was isolated from avocado, pine, deodar, ornamental Lawson cypress, Italian cypress, incense cedar, arbor-vitae, camellia, myrtle, and heather nursery stock in southern California nurseries. The significance of this is further indicated by lack of recovery of *P. cinnamomi* in root and soil samples from native vegetation in virgin soils of southern California, or in healthy avocado groves. It is probable that the occurrence of *P. cinnamomi* on ornamental Lawson cypress in Oregon and on heather in the San Francisco Bay region is the result of movement of the fungus on infected nursery stock or infested soil from southern California nurseries. Similar spread of this fungus has undoubtedly taken place from nurseries in other southern regions of the United States. The relative ease of prevention makes recognition of this form of dissemination a vital consideration in control of some soil-borne diseases.

Joint Sessions with the Mycological Society of America

Induced biochemical mutants in Venturia inaequalis. Boone, D. M., and G. W. Keitt. Biochemical mutants were induced in a line of *Venturia inaequalis* by treatments with nitrogen mustard (methyl-bis (beta-chloroethyl) amine) or ultra-violet light (2750 A.). Screening techniques were adapted from well known methods that had been developed in similar studies with other microorganisms. Mutants with specific deficiencies for choline, inositol, purines, pyrimidines, histidine, arginine, and reduced sulfur, respectively, were obtained. The inheritance of the deficiencies for the following chemicals was studied: Choline, uracil, purines (3 mutants), histidine (3 mutants), arginine, and reduced sulfur. Each deficiency appeared to be single-gene controlled. The data indicated that some of these mutant genes can be located with reference to their respective centromeres on the basis of percentage of second-division segregation. Experiments to determine possible effects of these deficiencies on pathogenicity are in progress.

Marker races useful in studying survival ability of races of Puccinia graminis tritici. Bromfield, Kenneth R. and James W. Broyles. The relative survival ability of races of wheat stem rust was studied in mixtures of known composition transferred through several uredial generations on Little Club wheat. Composition of the mixtures was determined each generation by inoculating differential varieties. In mixtures involving race "orange 11," pustule counts were made both on Little Club and on the differential varieties. There was little agreement between ratios based on pustule counts on differential varieties and on the Little Club, except when the same pustule types were produced on both, or possibly when conditions for rust were optimum. Certain varieties consistently had more pustules than others, the difference depending somewhat on pustule type. Interactions between races and varieties were apparent. It was found that the difference in survival ability between any 2 races could be predicted from the behavior of each in mixture with a third race. No antagonism between races was observed; therefore it appears that the relative survival ability of many races can be determined by comparing races in mixture with 1 or more marker races.

Mutation in the incitant of leaf rust of wheat, Puccinia triticina. Brown, A. M. In November, 1951, a single, light yellow uredial pustule appeared in a culture of race 15 of leaf rust of wheat that had been carried through 4 generations in the uredial stage in the greenhouse. From this pustule a weak strain of race 15 was eventually identified. The aberrant strain required a week longer than the parent race to reach its full development in the uredial stage. Its urediospores were less viable than those of the parent race and germinated tardily. Little Club wheat was moderately resistant to it and this condition made it difficult to obtain sufficient urediospores to inoculate the standard differential hosts. The rust does not survive storage conditions for more than a month and so can be maintained in culture only by periodic transfer to new hosts. As this aberrant strain of rust appeared in the late fall when conditions made it unlikely that a contamination came from sources outside the greenhouse, it is thought that it originated by mutation from urediospores of race 15. Strains of leaf rust of wheat with yellowish colored urediospores have appeared in cultures originating from aeciospores produced by artificial inoculations of *Thalictrum glaucum*, but the absence of a known aecial host in western Canada seems to exclude the possibility that the aberrant strain of race 15 originated by sexual reproduction.

Observations on the growth of Uromyces caladii in tissue cultures of Arisaema triphyllum. Cutter, Victor M., Jr. (No abstract submitted.)

Notes on Physalospora ilicis (Schleich. ex Fr.) Sacc. Driver, Charles H. (No abstract submitted.)

Some problems of species concept and nomenclature in the smut fungi. Fischer, George W., and Charles Gardner Shaw. Numerous cases still exist of delineation of species of smut fungi on a host basis. Morphologic distinction of such species is uncertain at best because they represent only minor variations. Such a species concept results in a multiplicity of binomials where a few would serve the purposes of taxonomy. As a compromise between a strict morphologic concept (which could result in consolidation of species obviously unrelated) and a liberal physiologic concept (which, carried to the ultimate application, could give specific rank to every physiologic variant of any kind) a species concept is proposed for the smut fungi which would recognize host specialization at the family level. Thus, species of similar morphology and symptomology having hosts in the same plant family would be consolidated into recognizable morphologic species under the oldest binomial permitted (and dictated) by International Rules of Botanical Nomenclature. This procedure sometimes results in allegedly illogical binomials (e.g. *Ustilago hordei* for covered smut of oats) on the false assumption that a specific epithet derived from a host genus name implies specialization to that genus. However, phytopathological literature and practice, in general, is replete with widely used and accepted binomials that are similarly derived and "illogical."

Inheritance of the sexes and compatibilities in fungi. Hansen, H. N., and William C. Snyder. In the homothallic, hermaphroditic fungus *Ceratostomella fimbriata* 2 kinds of mutants occur both of which are self-sterile. One of them produces perithecial fundaments and is herein designated as "female." The other produces no fundaments and is designated herein as "male." The female can be fertilized by the normal, self-fertile hermaphrodite and also by the self-sterile male, but it can not fertilize other females or hermaphrodites. The male can fertilize both females and hermaphrodites, but it can not be fertilized. From the cross female X hermaphrodite are obtained females and hermaphrodites in a 1:1 ratio. From the cross hermaphrodite X "male" are obtained hermaphrodites

and males in a 1:1 ratio. Results of these two crosses would seem to show that hermaphroditism is governed by a single factor and that the factors for female and male are allelic to it. This is not the case, however, for from the cross female X male are obtained self-fertile hermaphrodites, self-sterile females, self-sterile males and completely sterile neuters, indicating that the factors for female and male are not alleles but are inherited independently and that when they both occur in the same thallus the condition known as hermaphroditism exists. In the heterothallic, hermaphroditic fungus *Hypomyces solani f. cucurbitae* it was found that the factors for compatibility were inherited independently of those for female and male and the presence of the compatibility factors in the neuters was demonstrated.

Mutation and environmental selection of growth types in Ustilago zeae. JACKSON, ELSA L. AND J. B. ROWELL. Among monosporidial haploid lines of *Ustilago zeae* there are strict mycelial, strict sporidial, and intermediate types. The growth type of the latter can be shifted by nutrition. Mutation in monosporidial lines can also cause changes in growth types, as shown by studies of a strict mycelial line, 17D4-C7. In liquid culture this line produces different kinds of sporidial and mycelial mutants; on solid media only mycelial mutants appear because sporidial mutants tend to be obscured. In liquid culture, on the other hand, cells of sporidial mutants become appreciably numerous after about 10^{12} divisions, and after 10^{27} divisions 97 per cent of the colonies in platings from the liquid are sporidial. As the mycelial types tend to form hyphal aggregates, the predominance of sporidial types in dilution plates does not necessarily indicate that they are more numerous or prolific. On agar media the mycelial types tend to outgrow the sporidial types. Sporidial and mycelial mutants may in turn produce both mycelial and sporidial mutants.

Additional gene pairs conditioning pathogenicity in Venturia inaequalis. KEITT, G. W., AND D. M. BOONE. Three more gene pairs for conditioning pathogenicity in *Venturia inaequalis* were found, bringing to 6 the total number of gene pairs now known, respectively, to condition pathogenicity of wild-type lines of this fungus to leaves of an apple variety or group of varieties. These 3 gene pairs are tentatively named Haralson_2, Hyslop, and Grimes Golden, each after the first differential variety found for it. In each case, with a differential apple variety one allele conditions typical sporulating lesions and the other conditions flecks with sporulation lacking or sparse. Each of these 3 gene pairs was studied genetically to find whether the map distance of its locus from the centromere could be determined on the basis of percentage of second-division segregation. The available results indicate that this can be done only for Haralson_2. The results from the 8 isolates from each of 43 asci indicate that the Haralson_2 locus is approximately 12 cross-over units from its centromere.

The behavior of mutable black lines of Ustilago zeae. LU, SHIH I., E. C. STAKMAN, AND J. B. ROWELL. Colorless mutants appeared with mutation rates of 0.1 per cent or more, in certain monosporidial black lines of *Ustilago zeae*, and often tended to dominate the original black colony. Many colonies of diverse color appeared in dilution plates from sporidial suspensions of young, homogenous black colonies. In liquid shake cultures the ratio of white to black cells increased linearly by a factor of 0.13 per cent with each 10-fold increase in number of cells of 1 black line during logarithmic growth. This increase apparently was due to the frequency of mutation and not to differential growth rates or antagonism between lines. A mutation rate of 0.16 per cent was calculated by plating all the progeny from individual cells of this line. Several black mutants induced by exposing the relatively stable vinaceous line 10A4 to polonium behaved similarly. Back mutation to black also occurred among the colorless mutants, but at a lower rate. The black pigment results from oxidation of phenolic compounds by tyrosinase and its formation is inhibited by ascorbic acid.

Nuclear cytology and morphologic variation in Helminthosporium carbonum Ullstrup. ROANE, CURTIS W. The nuclear condition of mycelium, conidiophores and conidia of *Helminthosporium carbonum* race 1 was determined following a modified Feulgen reaction. The mycelial cells were uninucleate, binucleate, and multinucleate; the multinucleate condition was most common. Nuclei are apparently free to be carried in the protoplasm flowing from cell to cell. Conidiophores arose from all types of mycelium. At first their cells were uninucleate or binucleate; later they became multinucleate. During sporulation several nuclei flowed into each conidium from the conidiophore. As the conidia enlarged, the nuclei number increased so that each cell of the 1- to 9-celled mature conidia contained from 1 to 8 nuclei. Upon germination only the polar cells functioned. The germ tubes were multinucleate from the beginning and nuclei were observed in the very tips. It was concluded that if heterocaryosis occurs in this fungus the condition would be maintained in its life cycle. From 3 to 12 conidia were hand picked from each of 26 conidiophores and resulting colonies cultured on 5 differential media. Within conidiophore families, nearly all colonies were morphologically distinct on each medium. It is concluded that *H. carbonum* is heterocaryotic and that heterocaryosis in this fungus may be perpetuated indefinitely.

A method of preparing fungi for cytological studies. ROANE, CURTIS W. A modification of DeLamater's Method, using cellophane squares placed on the surface of suitable agar media in culture plates, has greatly facilitated the study of nuclear cytology of *Helminthosporium carbonum*. The squares were boiled to remove wax and grease, then 3 sterilized 1-in. squares were arranged in a triangle and the inoculum was planted in the center of the triangle. After the mycelium had grown over the surface of the cellophane, the latter was removed from the plates and plunged into a fixative. Mycelium, conidiophores, and young conidia remained undisturbed, but most of the mature conidia were detached by the fixative. Small quantities of the staining materials were placed in Syracuse dishes and the cellophane bearing the fungus was manipulated through stains with forceps. Upon completion of the staining schedule, the squares were quartered and mounted in "Permount" under No. 0 cover slips. The cover slips were kept under pressure until the mounting medium hardened. Spore germination was studied by spreading spore suspensions over the cellophane and fixing after different intervals of time.

Studies on the lyophilization of Puccinia Uredospores. SHARP, E. L. AND F. G. SMITH. Lyophilization has been shown to be an effective means of preserving collections of *Puccinia* species. Lyophilized uredospores of *P. coronata* when properly assayed have exhibited no significant drop in viability after more than 2 years in storage and have given heavy infection on susceptible oat varieties. Limited work with other species of *Puccinia* has given similar results. Races 57, 88, 98, and 101 of *P. coronata* have shown no change in mean infection type ratings on the differential oat varieties after lyophilization and storage. For use as inoculum lyophilized spores may require moisture equilibration before application to the host plant. Further study of the lyophilization and storage conditions have shown that, (a) small quantities of spores (cir. 20 mg.) can be processed in as little as 15 minutes, (b) larger quantities (up to 500 mg.) for field inoculations require no more than 2 hours, (c) efficient removal of both oxygen and moisture is essential for long storage life, and (d) lyophilized spores can be stored for a least 6 months at temperatures as high as 35°C.

Comparative efficacy of male and hermaphrodite in the fertilization of perithecial primordia. SNYDER, WILLIAM C., AND H. N. HANSEN. Receptive perithecial primordia of a heterothallic form of *Hypomyces solani* were fertilized by

the application of mixtures of conidia from a compatible male and a compatible hermaphrodite. Single ascospore analyses of the resulting perithecia have indicated that the male thallus is several times more efficacious as a fertilizing agent than the hermaphrodite.

Nutritive value of rust-infected bean leaves. YARWOOD, C. E., ALICE HALL, AND MARJORIE NELSON. The habits of snails, slugs, and certain insect larvae in eating out rusted areas of leaves in preference to healthy areas, suggested that rust infection had some unique effect on the nutritive value of leaves. Microbiological assay of healthy and rusted bean leaves for several growth factors showed that rusted leaves contained about 15 times as much pantothenic acid as healthy leaves and the uredospores of the rust (*Uromyces phaseoli*) contained more pantothenic acid per unit of dry weight than did the diseased tissue containing host and fungus. Snails (*Helix asper*) confined with rusted bean leaves having 1.5 mg calcium pantothenate per gram of leaves, gained weight faster than snails confined with normal bean leaves. Rats (*Rattus norvegicus*) on a pantothenate-deficient basal diet, gained weight faster when given a supplement of rusted bean leaves than when given a supplement of healthy bean leaves.

Joint Session with the Potato Association of America

Meteorological data correlated with potato and tomato late blight in northeastern United States. HYRE, R. A. During 3 growing seasons, 1949-1951, hygrothermographs were located in 7 potato fields, 4 tomato fields, and in 1 *Phytophthora infestans* inoculated tomato plot in Delaware, Maine, Maryland, New York, Ohio, and Pennsylvania. Local pathologists serviced the instruments and supplied late blight records. Precipitation was secured from Climatological Data. In analyzing the data, 3 sets of climatic conditions were considered to favor blight: 1) average, or greater, rainfall with average temperature below 75° F.; 2) 10 or more consecutive hours of temperature 70° F. or less and relative humidity 95 per cent or more; and 3) 48 or more consecutive hours of temperature greater than 50° F. and relative humidity greater than 75 per cent. With the 3 methods, good correlation occurred in the following percentage of cases: 1) "rainfall-temperature," 92; 2) "10 consecutive hours," 75; 3) "48 consecutive hours," 42. There was no correlation in 8, 25, and 50 per cent of the trials, respectively. In the remaining tests, correlation occurred during part of season. The "10 hour" method was least accurate when rainfall was deficient. This was illustrated at State College, Pennsylvania, during August, 1951, when there were 26 "favorable" periods yet blight spread to only a few plants in 4 rows from the original infection. In this case, rainfall was 56 per cent deficient during both July and August.

Yearly D-D treatments and continuous potato production in relation to the golden nematode population of the soil. MAI, W. F., AND BERT LEAR. The viable golden nematode population of a heavily infested field plot was decreased 99 per cent by fall application of 450 lbs. per acre of D-D mixture to a depth of 4 inches on 10-in. centers by a hand applicator. The number of living golden nematodes was kept at a low level for 4 years by yearly D-D applications, although potatoes were grown each year. Viability was determined by examination of larvae under a microscope and by determining the number of new individuals developing on potato roots. In soil samples taken after treatment the number of cysts containing living larvae per 2 oz. of soil for the 4 successive years were: .08, .33, 0, and 0. A few immature females were present on potato roots each year. In each of the 4 years yields of Green Mountain, a late maturing variety, and Cobbler, an early maturing variety, were significantly higher on soil treated with D-D than on untreated soil.

Evaluation of the phloroglucinol test for the diagnosis of potato leafroll. NATTI, JOHN J. Three methods of potato leafroll diagnosis—observation of external symptoms, observation of internal symptoms by means of the phloroglucinol test, and aphid-transmission tests to indicator plants—were each applied to the same potato plants. The plants were grown in the greenhouse and originated from tuber lots containing both infected and noninfected tubers. A total of 964 plants belonging to 6 named varieties and 5 unnamed seedlings were tested. Virus was transmitted by aphids from 307 of these, but only 237 showed external symptoms and 217 gave positive phloroglucinol tests. With Katahdin, Sebago, Ontario and an unnamed seedling, the phloroglucinol test was more reliable than diagnosis by observation of external symptoms. In tests with Snowdrift, Kennebec and Essex, these 2 methods gave similar results. With some of the unnamed seedlings, diagnosis by the phloroglucinol test was strikingly inferior to diagnosis by external symptoms. In general, infected plants which did not show internal symptoms did not show external symptoms. Results indicate that diagnostic methods based on the reaction of reagents with virus-infected tissues or products of infected tissues are not effective until infection has progressed beyond a certain minimum stage.

Natural barriers protect potatoes from soft rot infection. SMITH, WILSON L., JR. AND HELEN F. SMART. Potatoes were stored 1, 3, and 6 months at 40° F. Slices from these were then held in moist chambers 1 to 4 days at 80°, 70°, 60°, 50° and 40°F. Microscopic examinations of sections of these showed that at all temperatures suberin and periderm development were slower when tubers were stored 3 or 6 months than when they were stored 1 month. In 4 days development wos most rapid at 80° or 70°; suberin developed less rapidly and periderm was barely apparent at 60°; slight suberization, but no periderm developed at 50° and 40°. Varieties differing in capacity to form protective barriers were ranked in the following descending order: Irish Cobbler, Kennebec, Katahdin, Teton, Sebago, and Mohawk. When slices of each variety from all temperatures were inoculated with soft rot bacteria and placed at 70°, infection was abundant unless several layers of suberized and periderm cells had developed. No infection occurred in slices previously held 3 days at 80° or 70°; infection was less in those held 4 days than in those held 1 day at 60°; in those held at 50° or 40° infection was not decreased. Infection decreased with varietal capacity to form protective barriers.

The appearance and survival of new races of Phytophthora infestans. THURSTON, H. D. AND CARL J. EIDE. In September, 1951, a few large leaf lesions of late blight were found in 2 fields of Cherokee potatoes in Minnesota. In greenhouse tests isolates from these lesions produced heavy infection on several clones deriving blight resistance from Muller's "W" races or *Solanum demissum*. Numerous previous isolates from Minnesota caused flecks or small lesions on vigorous tissue and normal lesions only on senile leaves of these clones. Pathogenicity did not increase when the same clones were reinoculated with sporangia from each type of lesion. Similar adaptation experiments during the past 3 years were negative. Therefore it is suggested that races like that found on Cherokee may result from occasional genetic changes and become abundant only on a selective variety. Cobbler plants were

inoculated with a mixture of approximately equal numbers of sporangia of the Cherokee isolate and an ordinary field isolate. After 3 successive transfers to Cobbler, both Cobbler and Cherokee plants were inoculated. There were 20 times as many lesions on Cobbler as on Cherokee, indicating lack of survival ability of the Cherokee isolate in mixtures. This may explain why new races might not be found until the selective variety is present in considerable numbers.

Field studies on the development and survival of Phytophthora infestans sporangia on potato and tomato plants. WALLIN, J. R. In field experiments *Phytophthora infestans* sporangia appeared on potato leaflet lesions 14 hours after they were brushed free of sporangia. During 6 of the 14 hours temperatures were 70° F. or less, and humidities were 90 per cent or more. On the same set of leaflets sporangia became increasingly abundant from 8:00 A. M. until 4:00 P. M., although the air temperature reached 89° F. during this period. On another set of potato leaflet lesions brushed free of spores, sporangia appeared after 13 hours at 70° F. or less and humidities of 90 per cent or more. Spore viability was 4 per cent for sporangia from blighted potato and tomato leaflets exposed 5 hr. to temperatures of 70° F. or less and humidities of 90 per cent or more. Twelve and 17 per cent viable sporangia were obtained from potato and tomato leaflet lesions, respectively, exposed 12 nocturnal hr. to relative humidities above 90 per cent and temperatures above 70° F. On 3 afternoons, with air temperature maxima of 86°, 85°, and 92° F., viable sporangia were collected from potato and tomato lesions. Of 5 evening collections yielding viable sporangia, 3 were made after day temperatures of 77°, 83°, and 85° F. The viability of sporangia collected at 1:30 P. M. and 8:00 P. M. indicated that potential inoculum on leaflets survived diurnal adverse temperatures and humidities.

Forecasting late blight in Indiana from mean temperature and cumulative rainfall data. WALLIN, J. R. AND R. W. SAMSON. The mean temperature and amount and frequency of rainfall were compiled for 3 stations in southern and 4 stations in northern Indiana from June 1 to September 13 over a 37 year period, 1914-1951. The 2 sections were considered separately because of their distinct weather-blight histories. Seven blight years were recorded for southern, and 12 for northern Indiana. Straight lines were fitted to the weekly cumulative rainfall data for each year and a "critical rainfall line" was established midway between the severe and no-blight year lines. The "critical temperature line" was considered 75°F. Rainfall for the individual years was considered favorable for blight if the cumulative weekly amount or frequency equalled or exceeded that of the critical line, while temperature was considered favorable if the weekly mean was 75°F. or less. Hypothetical blight forecasts formulated from cumulative rainfall amount and weekly mean temperature were 56 per cent and 69 per cent correct for northern and southern Indiana, respectively. Blight forecasts for northern and southern Indiana based upon cumulative rainfall frequency and weekly mean temperature were 50 per cent and 61 per cent correct, respectively. These results suggest that other information in addition to rainfall and weekly mean temperature data is needed to predict effectively the development of the late blight pathogen.

Invitational Papers in the Symposium on Disease Resistance

Control of tobacco diseases through resistance—1912 to 1952. CLAYTON, E. E. The modern program of breeding disease-resistant varieties of tobacco began in Wisconsin. The sequence of significant developments was as follows: 1912-25, the development of black root rot resistant varieties; 1926-32, the first black shank resistant variety; 1933-40, mosaic immunity transferred from *Nicotiana glutinosa;* 1941-52, the first bacterial wilt resistant variety, wildfire immunity transferred from *N. longiflora,* blue mold resistance and black root rot immunity transferred from *N. debneyi.* Some of the outstanding disease resistant varieties released have been Havana 142, Kentucky 16, Rg, and Oxford 26. Progress has been made in the last decade in combining in one variety resistant factors for 2 or more diseases. Resistance obtained within the cultivated species, *N. tabacum,* has proven moderate in degree and of polygenic inheritance. High level resistance and in 3 cases simple monogenic dominant immunity, has been obtained from the *Nicotiana* species that are distantly related to *N. tabacum.* It appears that the cultivated tobacco can be greatly improved by gene transfers from at least 5 other *Nicotiana* species, and that such transfers may be accomplished without measurable effect on plant characters other than disease resistance.

Control of sugar beet diseases through resistance, 1918-1952. COONS, G. H. Breeding of sugar beet varieties resistant to curly top began in 1918 in California and to *Cercospora* leaf spot in 1920 in Colorado. U. S. 1, introduced in 1931, was followed by progressively more resistant curly top reselections (U. S. 34, U. S. 33, U. S. 12, and U. S. 22). The first leaf spot resistant variety, U. S. 217, released in 1937, was replaced by hybrids produced by mating highly-resistant inbreds (U. S. 200 × 215, U. S. 215 × 216, and U. S. 216 ×226). A successful variety must combine many factors; thus, winter plantings in California require a highly productive, high quality variety that is both curly top resistant and non-bolting. U. S. 15, introduced in 1936, was moderately resistant to curly top and was highly non-bolting, and in addition was highly resistant to downy mildew and rust, serious diseases in California coastal districts. For the humid area, leaf spot-resistant varieties are reselected against black root. For California, the new varieties, U. S. 56 and U. S. 75, combine non-bolting, downy mildew, and rust resistance with very high curly top resistance. The factors for resistance have been found in cultivated sugar beet but all *Beta vulgaris* forms seem susceptible to virus yellows, hence the search in wild species of *Beta* for genetic factors against this new disease.

Problems involved in breeding oats for disease resistance. MURPHY, H. C. The development and distribution of rust-, smut- and *Helminthosporium*-resistant oat varieties represent an outstanding contribution to American agriculture. About 90 and 75 per cent of the oat acreages of the Corn Belt and the United States, respectively, were first changed over to Victoria derivatives during 1941-45, and then to Bond derivatives during 1946-50. These acreages of resistant varieties had a striking effect on the prevalence and severity of certain diseases and biological races of the pathogens. *Helminthosporium victoriae* was discovered in 1944. By late 1946 and 1947 it had become a limiting factor for profitable oat production with Victoria derivatives in most of the United States. Previously rare or unknown races of stem rust and smut also attacked the Victoria derivatives. Victoria and Bond derivatives were resistant to 98 per cent of all crown rust collections in the United States previous to 1944, but by 1950 the Bond derivatives were susceptible to 98 per cent of all collections. Most Bond derivatives were susceptible to race 7 of stem rust which became more prevalent, starting in 1950. Varieties resistant to *H. victoriae,* to all races of rust and smut known to be present in North America, and to some minor diseases, are now being developed. With an increase in

acreage of these new varieties additional diseases and races doubtless will appear.

Inheritance of disease resistance in the potato. STEVENSON, F. J. Immunity to the leafroll virus and to viruses A, X, and Y to late blight, to common scab, and to hopperburn is found in the wild species of potato. *Solanum tuberosum* carries immunity to viruses A and X and net necrosis and resistance to virus Y, leaf roll, yellow dwarf, late blight, scab, *Verticillium* wilt, ring rot, and hopperburn. Studies of the wild species of potato have been increased. These species have much to contribute to genetic programs, but the breeding work is mostly concerned with *S. tuberosum*. Genetic hypotheses have been proposed for immunity in this species to wart, viruses A and X, late blight, and for resistance to scab. Resistance to other diseases is heritable; the number of genes is undetermined. Factors that complicate the gentic problem are irregular segregations because of irregular chromosome behavior, the lack of standardized tests, environmental effects, and biological races of the pathogen in question. Varieties that are resistant to 1 or more of the following diseases have been released: Mild mosaic, leaf roll, net necrosis, yellow dwarf, scab, late blight, and ring rot. One seedling variety is immune to viruses A and X and the common races of late blight. It is also resistant to common scab and *Verticillium* wilt. Resistance to late blight in several varieties was inherited from the wild species *S. demissum*.

Invitational Papers in the Symposium on Physiology of Parasitism

Toxins and tissue respiration. ALLEN, PAUL J. Disturbances of energy metabolism early in the course of infection have been recognized as changes (usually increases) in temperature, O_2 uptake, CO_2 production and enzyme activity. In many instances these changes are clearly attributable to diffusible substances (toxins) passing from the parasite to the host. The nature of these toxins is considered, and their action on respiration is discussed in the light of current knowledge concerning poisons and cell metabolism. Possible benefits to the parasite resulting from the altered host respiration are pointed out. The effects on respiration of toxins from higher plants and other sources are reviewed. Recent developments in the study of metabolic regulation and metabolite antagonists may provide a useful basis for working hypotheses in the investigation of parasitism. Competition between toxins of one organism and normal metabolites (either activators or suppressors) of another may be an important process affecting the rate and course of energy conversions and hence affecting the welfare of the organisms concerned.

Defense reactions of plants to the presence of toxins. BLOCH, ROBERT. Outstanding problems of physiological specificity in plants are the nature of hormones and their action, of cellular determination, and of host-parasite relationships and disease resistance. In the last named group, the defense mechanism of the host against pathogenic toxins is of particular interest. Extracts containing the toxins induce the same specific disease symptoms as presence of the pathogen itself. But in both cases the defense reactions do not possess the specificity of those in the animal body; they consist in demarcation and localization of toxin-containing areas rather than in direct destruction and biochemical neutralization of the toxins. The toxins have an injurious effect on cellular metabolism, but little is known about the chemistry of the process. The injurious effect often results in death of cells and in defense reactions comparable to those in non-parasitic wound reactions. After a necrogenous phase of cellular degeneration and more or less pronounced autointoxication by decomposition products of the host cells, infiltrating healthy cells and ducts, meristem formation follows which, in favorable cases, delimits the diseased area. These natural defense reactions of the plant to the presence of toxins are at any rate indirect and incomplete, but something may be learned about the mechanism of counteracting the pathogenic toxins more directly from recent advances in internal plant chemotherapy.

An approach to the physiology of toxin formation in microorganisms. HOLLIS, J. P. Suitable biological material for testing the hypothesis that toxins and antibiotics are produced as shunted compounds in a metabolic system by plant pathogens, when they come into contact with unusual substrates such as plant surfaces, includes species of *Rhizoctonia, Sclerotium, Phymatotrichum* and *Streptomyces*. The presence in *S. scabies* of a shunt melanin-indicator system of amino acid metabolism makes it possible to test whether inter-relations exist between this system and metabolic shunts of toxins and antibiotics. This organism, in the presence of tyrosine in high concentration, an inorganic nitrogen source such as KNO_3, favorable pH and stimulatory amino acids, converts more than 50 per cent of the tyrosine to melanin during a 4 day shake culture period. Amino acids stimulating melanogenesis in all strains tested include arginine, lysine, histidine, phenylalanine, alanine, aspartic acid and glutamic acid. Melanin-stimulation patterns differ in pathogenic, nonpathogenic and scab lesion surface isolates of *Streptomyces*. A principal question which may be resolved with this system is whether toxin formation is adaptive or constitutive. It is considered that similar indicator shunt systems can be developed in other pathogenic microorganisms.

Detection of microbial toxins by the use of radioisotopes. WHEELER, H. E. Present evidence regarding the role of microbial toxins in plant disease has been derived chiefly from observations of disease symptoms in host tissues not occupied by the pathogen and from studies of host reaction to toxic agents produced by plant pathogens in artificial culture. More direct evidence might be obtained if methods were available for detecting very small quantities of toxin and for distinguishing toxic materials produced by the pathogen from those released by the break down of host cells. Radioisotopes, used as tracers, offer a tool for the development of such methods since extremely minute quantities of materials labeled by the use of radioisotopes can be detected.

Invitational Papers in the Symposium on Plant Parasitic Nematodes

Ectoparasitic nematodes of plants. CHRISTIE, J. R. Of the many ectoparasitic nematodes that injure the roots of plants, at least 4 are pests of major importance in the southeast, namely, *Belonolaimus gracilis* Steiner (the sting nematode), *Trichodorus* sp. (a stubby-root nematode), *Dolichodorus heterocephalus* Cobb (the awl nematode), and *Xiphinema americanum* Cobb (a dagger nematode). All 4 feed mostly at root tips and all produce somewhat similar symptoms. Each feeds on many different kinds of plants and, at present, the host lists are far from complete. *B. gracilis* probably causes greater crop losses in Florida than any other plant parasitic nematode. Corn, celery, bean, cowpea, and strawberry may be very severely injured; peanut, pepper, cotton, soybean, and squash may be more or less severely injured. *Trichodorus* sp., which is widespread in the south, probably attains its greatest impor-

tance as a parasite of corn. Other crops that may be severely injured include celery, beet, cabbage, chayote, squash and tomato. *D. heterocephalus* is very much like the sting nematode and, while not so common or widespread, can be equally devastating. Crops that may be severely injured include corn, bean, celery and tomato. *X. americanum* is important primarily as a parasite of trees and shrubs although it feeds on the roots of other kinds of plants. Severe injury may be inflicted to the roots of oak trees (*Quercus laurifolia*), pecan trees, and azaleas. Although found occasionally around the roots of oat, corn, and some of the grasses, this nematode is not common in land devoted to the cultivation of annual crops.

Methods of assaying nematicides. McBeth, C. W. The search for new nematicides includes: (a) primary screening of hundreds of chemicals of unknown biological activity and the testing of those compounds found to have inherent toxicity to nematodes on different species of nematodes under different soil conditions and on different crops. Primary screening is done mostly by a few commercial laboratories which have developed specialized and satisfactory methods. The secondary testing is also carried on by commercial laboratories as well as experiment stations and other workers. Primary screening work generally includes: (a) an exposure of a standard nematode culture to water solutions or suspensions of test chemicals of known concentration, (b) soil application of fumigants where the test material is injected into the center of a standard container of soil and nematode kill determined after a holding period under constant conditions and, (c) a mechanical mixture in soil of test chemicals of low volatility. In secondary testing the chemicals must be evaluated by various methods using applications which would be dependent upon their volatility, water solubility, and chemical stability in contact with air and soil. Reaction of various crops growing in nematode infested soil are checked first in the greenhouse and later in small field plots. Finally, such related problems as phytotoxicity, vertebrate toxicity, and chemical residues in soils and plants, are examined.

Methods of detecting and investigating plant-parasitic nematodes. Raski, D. J. The diagnosis of nematode infections under field conditions is usually very difficult or impossible due to the small size of these animals and their intimate association with the soil. Plant symptoms are often unreliable because they are not specific for nematodes in many cases. For this reason many nematode problems have not been correctly diagnosed and increasing attention should be given to the possibility of nematodes as causes of many unsolved problems of poor growth or decline of plants. Laboratory examination and identification are essential before positive diagnosis can be made. Differential fumigation and host plants are useful in detecting and investigating nematodes if their limitations are understood. The practice of making permanent collections, on slides or in formalin, is strongly urged in any work involving nematodes, or where they are even suspected of being a factor in experimental work. The final identification of nematodes should be made only by specialists trained in nematology.

Phenomena and problems of the internal parasitism of nematodes in plants. Steiner, G. The mechanism of location of a plant host by nematode parasites is discussed. Included are the nature of attraction and scent of host, receptors in parasite, mode of migration to and invasion of host by mechanical and chemical action, location of exact source of food, emplacement. Adaptive relations of parasite to host are described, including morphological, physiological, developmental and propagative modifications. Types of parasitism, such as tissue versus cellular parasitism, sedentary versus migratory parasitism, vascular versus parenchymal parasitism, also root, stem, leaf, flower and seed parasitism in their significance for parasite as well as host are considered. The metabolism of the parasite in its relation to the host including extra oral digestion in certain groups, effects of nutritional deficiencies on the host, necrosis of tissues, toxicity, gall and tumor formation, nanism and gigantism is also discussed. Internal parasitism of nematodes in plants is compared with that in animals and discussed as a problem of plant therapy.

Progress and prospects in the chemical control of nematodes. Taylor, A. L. The history of soil fumigation for nematode control is reviewed, with emphasis on the difficulties encountered in commercial development. The economic aspects of soil fumigation are discussed, as is the educational program necessary in introducing new products and procedures. The future development of soil fumigation is estimated to be one of steady, rather than spectacular, growth.

GEORGE JAMES WILDS, JR. 1889-1951

C. H. Rogers

GEORGE JAMES WILDS, JR.
1889—1951

Dr. George James Wilds, Jr., died at Columbia, South Carolina, October 26, 1951. He is survived by his widow, Ruth Lawton Wilds, residing at their home in Hartsville, South Carolina, and his son, George James Wilds III, a student at Clemson College.

Dr. Wilds was born at Longtown, Fairfield County, South Carolina, September 19, 1889. In 1908, he moved to Hartsville where he became associated with the late David R. Coker in field-crop breeding. He was graduated from the University of South Carolina in 1913 and received the Master's degree from Cornell in 1917. In the interim he continued as plant breeder with Coker's Pedigreed Seed Company, became Director of Plant Breeding in 1919, and in 1938 was appointed President and Managing Director, a post he held at the time of his death.

Dr. Wilds was recognized as the south's foremost plant breeder and was widely known for his contributions to southern agriculture through the breeding of high-yielding, early-maturing, Fusarium-wilt-resistant, short-staple upland cotton of greater length, as well as disease-resistant oats and wheats of superior qualities. Among the outstanding varieties developed by Dr. Wilds, which are grown throughout the South, are Coker 100 Wilt and Coker-Wilds (staple) cottons, Victorgrain and Fulgrain oats, and Redheart and Hardired wheats.

He was the author of numerous articles on crop breeding and development in the south, especially with reference to cotton and small grains. His articles appeared in southern farm journals and as proceedings of meetings of southern agricultural organizations, including crop improvement associations, state agronomy societies, and the Association of Southern Agricultural Workers.

Dr. Wilds was awarded a testimonial for distinguished service to the agricultural development of South Carolina by Clemson College in 1932; a medallion by the Association of Southern Agricultural Workers for "years of distinguished service" to southern agriculture in 1947; and in 1948 was given the South Carolina American Legion distinguished service award.

In 1947, he was recognized by The Progressive Farmer as "Man of the Year in Service to South Carolina Agriculture." The Progressive Farmer said of him in part: "Dr. George Wilds has made life better for thousands here in the south by giving them means for improving their conditions and by bringing to them inspiration and vision."

He was given the honorary degree of Doctor of Science by Clemson College in 1937 and an honorary Doctor of Laws degree by the University of South Carolina in 1946.

He was a member of the American Association for the Advancement of Science, South Carolina Academy of Science, American Society of Agronomy, American Phytopathological Society, South Carolina Seedsman's Association (President 1947), Association of Southern Agricultural Workers, the Darlington County Agricultural Society (President 1945-47), Omicron Delta Kappa, Phi Beta Kappa, and Sigma Xi. He was a Mason, a member of the Hartsville Rotary Club, and an Elder in the First Presbyterian Church.

MORPHOLOGICAL AND PATHOLOGICAL STUDIES OF RACES 2, 7, AND 8 OF OAT STEM RUST [1]

M. K. Hingorani [2]

The composite nature of *Puccinia graminis* Pers. was first demonstrated in 1894 by Eriksson (2), almost a century after it was described by Persoon (6, 7) as a single morphological species attacking all the common cereals and grasses. Eriksson and Henning (3) divided the old *Puccinia graminis* into 2 species, *Puccinia graminis* (black rust) with aecidia and *Puccinia phleipratensis* (timothy rust) without aecidia. They further subdivided *Puccinia graminis* into several distinct, physiologically specialized strains, each limited in infection capabilities to a definite and distinctive group of cereals and grasses. They recognized the following sub-divisions and designated them as formae speciales: *avenae* on *Avena sativa; secalis* on *Secale cereale, Hordeum vulgare,* and *Triticum repens; tritici* on *Triticum vulgare; airae* on *Aira caespitosa; agrostis* on *Agrostis* spp.; *poae* on *Poa compressa;* and *epigaei* on *Calamagrostis* spp.

In the United States, Stakman and Piemeisel (9) isolated the strains *tritici, secalis, avenae, agrostis, poae,* and *phleipratensis* and designated them as biologic forms of *Puccinia graminis.* Later, Stakman and Levine (8) showed that the differences between these biologic forms were not solely physiological, but that the forms also differed in the shape and size of their spores, particularly the urediospores. Forms were therefore raised to varietal rank.

Recently, Fischer and Claassen (4) collected 3 cultures of stem rust from *Avena fatua, Poa ampla,* and *Agropyron spicatum,* the urediospores of which did not conform to the established biometric constants of any of the varietal complexes of *Puccinia graminis.* On the basis of their host range, however, the cultures from *Avena fatua* and *Poa ampla* seemed best identified with the variety *Puccinia graminis avenae.* This raises a fundamental question whether the differences between some races of *Puccinia graminis avenae* are such as to give them varietal rank. Comparative morphological and pathological studies of races 2, 7, and 8 of *Puccinia graminis avenae* were, therefore, made.

MATERIALS AND METHODS.—The races of oat stem rust most commonly found in the United States in recent years, races 2, 7, and 8, were obtained from the Federal Rust Laboratory, St. Paul, Minnesota. A single isolate of each race was used, stock cultures being maintained on the susceptible Bond oats.

Urediospore dimensions for the individual races were determined by measuring length and width of 200 spores in each population. Normal spores from the superficial layer of mature uredia were measured, care being taken to select uredia in the same stage of development. The spores were mounted in hot lactophenol and not more than 50 were measured from the same microscopic field.

In the study of relationship of host to the size of urediospores, both susceptible and resistant varieties were used but the environment maintained was uniform and favorable. In the experiments on environmental effects on the size of urediospores, however, light and temperature were independently altered. Proper controls were maintained in each case.

The cereals and grasses tested were grown in 2-inch pots, 10-15 plants in each pot, in the greenhouse. Inoculum from heavily infected oat plants of the highly susceptible Bond variety was applied directly to the cereal and grass seedlings; rust readings were taken about 2 weeks after inoculation, when the pustules had reached full development. In some cases, when reactions were not clear, inoculations were repeated 3 times.

COMPARATIVE MORPHOLOGY.—Dimensions of urediospores of races 2, 7, and 8 of *Puccinia graminis avenae* produced under different temperature and light conditions and on different hosts are in Table 1.

TABLE 1.—*Mean lengths and widths of urediospores of the 3 races of Puccinia graminis avenae produced under different conditions*

Condition [a]	Race	Length in microns	Width in microns
Optimum	2	27.10 ± 0.16	15.08 ±0.10
	7	25.19 ± 0.18	14.19 ± 0.12
	8	27.22 ± 0.18	15.79 ± 0.92
Reduced temperature	2	25.10 ± 0.22	14.35 ± 0.12
	7	24.11 ± 0.16	13.01 ± 0.10
	8	25.47 ± 0.20	15.29 ± 0.07
Reduced light	2	24.65 ± 0.20	13.71 ± 0.13
	7	23.99 ± 0.16	13.21 ± 0.09
	8	24.79 ± 0.21	14.70 ± 0.11
Resistant host	2	23.98 ± 0.18	13.63 ± 0.09
	7	23.57 ± 0.17	13.34 ± 0.07
	8	24.10 ± 0.18	14.79 ± 0.11

[a] Optimum conditions included temperature of 25° C., normal sunlight, and a susceptible host (Jostrain). Reduced temperature was 18° C. Reduced light was one tenth of normal sunlight. Resistant hosts were Minrus for races 2 and 7 and Richland for race 8; other factors were optimum.

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This paper forms a section of a thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in 1947, while the author was a Fellow of the Watumull Foundation.

The author wishes to express his gratitude to the Watumull Foundation for financial aid and to Dr. E. C. Stakman, Chief of the Division of Mycology and Plant Pathology, University of Minnesota, for his guidance in this research. He is also grateful to Dr. Helen Hart, Professor of Plant Pathology, for correcting manuscript.

[2] At present the author is at the Indian Agricultural Research Institute, New Delhi, India.

There are significant differences between the mean urediospore lengths and widths of races 2 and 7 and 8 and 7 when produced under optimum environmental conditions, but there seems to be no appreciable difference between those of races 2 and 8. Stakman and Piemeisel (9) studied the comparative sizes of urediospores of different varieties of *Puccinia graminis* and found that the range for the variety *avenae* was 19.20—37.12 × 13.76—25.60. The urediospore size of the 3 races is within this range.

Stakman and Levine (8) and Levine (5) showed that environmental conditions influence spore size in *Puccinia graminis* to a considerable extent. Later, Bailey (1) also found that environmental conditions result in changes of spore size as great as, or even greater than, the differences between spores of different races of *Puccinia graminis avenae* produced under similar conditions. The present study corroborates the results obtained by previous workers. Reduced temperature, reduced light intensity, and a resistant host tend to decrease spore size and increase variability of the 3 races.

INOCULATION OF CEREALS AND GRASSES.—Fifty-nine varieties of oats, 23 of wheat, 22 of barley, and 1 of rye were inoculated with the 3 races. Races 2 and 7 did not infect any of the cereals other than oats, whereas race 8 weakly infected 2 varieties of wheat (Clarkan, Little Club), 3 of barley (Hokudo, Nassau, Peatland), and the rye (Rosen). Few uredia of race 8 appeared on these hosts, but infection types produced were: (0;) on Clarkan, 1 on Little Club, 1— on Hokudo, 1 on Nassau and Peatland, and 1++ on Rosen rye. None of the oat varieties were immune from the 3 races.

Forty-nine species of grasses, belonging to 16 genera, were inoculated to determine their relative susceptibility to the 3 races. In some cases, more than one collection of a given species was tested. Different strains within a species were obtained from the Minnesota Seed Testing Laboratory. The species that proved to be immune from the 3 races are not included in Table 2.

TABLE 2.—*The infection types produced by races 2, 7, and 8 of Puccinia graminis avenae on seedlings of grass species*

Grass inoculated	Infection type produced by race		
	2	7	8
Agropyron caninum (L.) Beauv.	0;	0;	0;
A. cristatum (L.) Gaertn.	0	0	1
Bromus tectorum L.	2+	2+	2
Dactylis glomerata L.	4	4	4+
D. glomerata (Commercial strain)	1 to 2	4	4
Elymus canadensis L.	0;	0	1
E. glaucus Buckl.	0	0	1
Festuca elatior L. (Strain No. 2)	1	2	3—
F. rubra L.	4	4	4
Koeleria cristata (L.) Pers.	4	4	4
Lolium perenne L.	0;	2	1
Phalaris arundinacea L.	1	0	2—
Poa nevadensis Vasey	3—	2	3

From these results, it seems unlikely that there is much difference in the reaction of different grasses to races 2, 7, and 8 of *Puccinia graminis avenae*, although the 3 races behaved somewhat differently on a few grasses. The writer tested 9 strains of *Dactylis glomerata*, 8 of which were equally susceptible to the 3 races, but 1 varied in reaction. The latter was highly susceptible to races 7 and 8 and only slightly susceptible to race 2. Similarly, 4 strains of *Festuca elatior* were immune from the 3 races and 1 was slightly susceptible. *Elymus canadensis*, *Phalaris arundinacea*, *Agropyron cristatum*, and *Elymus glaucus* also varied slightly in reaction to races 2, 7, and 8.

Thirty-eight species of grasses were immune from the three races: *Agropyron dasystachyum* (Hook.) Scribn., *A. inerme* (Scribn. & Smith) Rydb., *A. pauciflorum* (Schwein.) Hitchc., *A. repens* (L.) Beauv., *A. saxicola* (Scribn. & Smith) Piper, *A. spicatum* (Pursh) Scribn. & Smith, *A. trichophorum* (Link) Richt., *Agrostis alba* L., *A. perennans* (Walt.) Tuckerm., *A. retrofracta* Willd., *A. spica-venti* L., *A. tenuis* Sibth., *A. verticillata* Vill., *Bromus inermis* Leyss, *Deschampsia elongata* (Hook.) Munro, *Elymus flavescens* Scribn. & Smith, *E. jejunus* Rydb., *E. sibiricus* L., *E. striatus* Willd., *E. triticoides* Buckl., *E. virginicus* L., *Festuca elatior* L., *F. fallax* Thuill., *Hordeum bulbosum* L., *H. nodosum* L., *H. jubatum* L., *Panicum virgatum* L., *Phleum pratense* L., *Poa ampla* Merr., *P. arida* Vasey, *P. compressa* L., *P. juncifolia* Scribn., *P. palustris* L., *P. pratensis* L., *P. trivialis* L., *Sitanion hystrix* (Nutt.) J. G. Smith, *Sporobolus airoides* (Torr.) Torr., *S. cryptandrus* (Torr.) A. Gray.

Except for 2 cases, the present results agree reasonably well with those of Stakman and Piemeisel (9). They found that *Agrostis alba* and *Phleum pratense* could be weakly infected by *Puccinia graminis avenae*, whereas the writer found them to be immune from the 3 races tried. This is probably due to the fact that the grass strains and races used by them were different from those of the writer. Fischer and Claassen (4) report that in 1937 D. C. Smith collected a rust on *Poa nevadensis* which in culture was identified as *Puccinia graminis avenae* race 2 by M. N. Levine, Pathologist, U. S. Department of Agriculture, stationed at University Farm, St. Paul, Minnesota. The writer also found that *Poa nevadensis* was moderately infected by the 3 races.

DISCUSSION.—Comparison of the urediospore morphology of races 2, 7, and 8 with established biometric constants of the 6 varieties of *Puccinia graminis* shows that the 3 races are within the range of variation expected for the variety *avenae*. Statistically significant differences in the size of urediospores were found between races 2 and 7 and 8 and 7, but not between races 2 and 8. Urediospores of race 7 were somewhat more variable than those of races 2 and 8. It appears, therefore, that variability within *Puccinia graminis avenae* may be accounted for by the differences in races and by the variability of spores within a race. Moreover, it has been shown that significant fluctua-

tions may be induced in a single race by varying the environmental conditions; reduced light and temperature and a resistant host seem to be effective in this respect. Thus, it may be concluded that the morphological differences alone cannot be relied upon as a means of identifying the individual races; and there seems to be no reason for elevating any of these races to a varietal rank.

Parasitically, also, the 3 races are well within the limits previously set for *Puccinia graminis avenae*. Except for the fact that race 8 weakly infected some varieties of wheat barley, and rye, none of the 3 races produced any visible effect on cereals other than oats. Stakman and Piemeisel (9) have already shown that *Puccinia graminis avenae* could weakly, but consistently, infect barley and rye, but they did not get any infection on wheat varieties. These results were, therefore, not unexpected.

Testing 75 collections of 49 species of grasses to the 3 races of oat stem rust showed that the grass-host range of *Puccinia graminis avenae* is rather limited. Thirty-eight species of grasses tested were immune and 4 were slightly susceptible. Only 3 were completely susceptible to the 3 races. Within some species of grasses, different lines or collections differed somewhat in susceptibility. There were minor differences in the virulence of the 3 races on some grasses, race 8 being slightly more pathogenic than the other 2, but there is no evidence that the host range of the races on grasses is wider than that of the variety *avenae* as originally determined.

SUMMARY

Statistically significant differences in the size of urediospores were found between races 2 and 7 and 8 and 7 when grown under identical conditions, but no appreciable difference was observed between races 2 and 8. Urediospores of race 7 were somewhat more variable than those of races 2 and 8. However, the 3 races were within the range of variation expected for the variety *avenae*.

Significant fluctuations were induced in urediospore size of each race by varying environmental conditions. Reduced light and temperature and a resistant host were found to be important in this respect.

Except for the fact that race 8 weakly infected some varieties of wheat, barley, and rye, none of the races produced any visible effect on cereals other than oats. All the varieties of oats were, however, attacked by the 3 races and none seemed immune.

It was found that the grass-host range of oat stem rust was rather limited. It seemed unlikely that the 3 races differed greatly in their virulence on grasses, although there were some minor differences between them.

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METHYL BROMIDE FOR DISINFESTING BURLAP BAGS AND MACHINERY TO HELP PREVENT SPREAD OF GOLDEN NEMATODE OF POTATOES [1]

Bert Lear and W. F. Mai

Between potato crops living larvae of the golden nematode, *Heterodera rostochiensis Wollenweber*, remain in the soil enclosed in egg cases in protective cysts. Franklin (2) found many cysts containing larvae capable of attacking potatoes in soil which had not been cropped with potatoes or tomatoes for 8 years. As a result, soil adhering to farm machinery offers an excellent means of transporting cysts from field to field. Soil from infested fields clinging to burlap picking bags is another means of spread. The complete disinfestation of such items offers many problems. Use of steam generated by a "jenny" (oil-burning flash-steam generator), although the best means currently employed, is not wholly satisfactory. In the first place, it is impossible to do a thorough job on bulky machinery; secondly, burlap does not withstand steaming. After observing such operations and noting the admitted inadequacies, the authors set out to find a chemical which might be used for these operations. After testing many volatile chemicals, it was decided that methyl bromide offered the best chance of success.

MATERIALS AND METHODS.—*Types of Enclosures Used to Confine Methyl Bromide.* Although fixed enclosure such as vaults would serve for treatment of bags, a cover of flexible material would prove more satisfactory for treatment of variously shaped and sized machinery. In the following experiments 2 types of fixed enclosures and 3 types of flexible covers were used.

Fixed enclosures.—1) An open-head metal drum 3 ft. high, with a capacity of about 8 cu. ft. and provided with a tight fitting lid with rubber gasket, was used. The methyl bromide was introduced through a plastic tube leading through a rubber stopper in a ½-in. hole bored in the center of the lid. 2) A 112 cu. ft. metal lined box served as a vault enclosure. The door and ventilation ports were provided with rubber gaskets. Constant temperatures ranging from 30° to 100° F. were maintained by thermostatically controlled freezer plates on the ceiling and back wall and a fan type heater. An 8-in. fan fixed at the point of introduction of methyl bromide served to circulate the gas and to expel it at the end of the test. A volatilizer consisting of a 25-foot coil of copper tubing suspended in a water bath containing a 1000-watt immersion heater was attached on the outside of the chamber. The fumigant was measured from a 50-lb. cylinder by weighing and was passed through the volatilizer before entering the chamber.

Flexible covers.—1) Warp sateen fabric impregnated with ethylcellulose [2] was the first type of cover used. This material is very rugged but requires several men to place a large tarpaulin over a bulky piece of machinery. Care must be taken not to allow the liquid methyl bromide to come in contact with the cover since the ethylcellulose will be dissolved. Cost of this cover is about 15c per sq. ft.

2) An equally heavy cover is a fabric with a vinyl resin formulation fused into the fabric with infra-red rays.[3] This material is resistant to mildew, water, inorganic acids, alkalies, gasoline, grease and alcohol. It may be sewed or cemented. It is flexible except at temperatures below freezing when it stiffens and cracks if handled or folded. Price of this cover is estimated at 15 to 23c per sq. ft., depending upon the weight of fabric used.

3) A polyethylene film [4] was the third type used. The clear materials has a wall thickness of 0.004 in. It is pliable even at low temperatures and light in weight, hence it can easily be placed over bulky machinery. It is readily heat-sealable so long as the material does not come in direct contact with sealing jaws. Also, it may be sewed, and it is resistant to liquid methyl bromide, gasoline and oils. The estimated cost of this cover is 1½c per sq. ft.

Determination of Treatment Efficacies.—Whether encysted larvae are alive or dead is not easy to determine. O'Brien and Prentice (4) and Triffit (6) first reported that exposure of cysts to water leached through a pot containing a growing potato plant accelerates the emergence of the larvae from cysts. This method was used for all premilinary determinations of treatment efficacies. After the treatment period, the cysts were placed for 2 weeks at 78° F. in petri dishes containing 25 ml. of filtered leachings. The cyst-leachings mixture was then placed in a volumetric beaker and the total volume adjusted to 100 ml. While this mixture was in constant agitation from an electric mixer, aliquots of 5 to 10 ml. were transferred with a pipette to Syracuse watch glasses. The larvae in each aliquot were then counted with the aid of a stereoscopic microscope.

With the above method, presence of larvae will indicate treatments of no value. But the mere absence of larvae emerging from treated cysts does not necessarily indicate non-viability since many sub-lethal treatments, both heat and chemical, have been shown to induce inhibition or retardation of larval emergence (5, 7). Feldmesser and Fassuliotus (1) reported inhibition of emergence in cysts treated with methyl bromide at 4 lb. per 1000 cu. ft. This is approximately only 1/6 of the smallest dosage found effective in these tests. For this reason, additional tests were run

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[2] Product of Shane Manufacturing Co., Inc., Evansville, Indiana.

[3] Obtained from Borg and Company, Inc., Springfield, Mass.

[4] Product of The Visking Corp., Terre Haute, Indiana.

with the 2 selected treatments. With some treatments, weekly examinations of treated cysts exposed to leachings were made for 5 weeks. After each weekly examination, the cysts were removed from the mixture by sieving and added to fresh leachings. Dead larvae were much more easily detected after an interval of at least 3 weeks.

To check further on treatment efficacies, cysts were sieved from the mixture and dried, following their exposure to root leachings. Counted numbers of these cysts were opened beneath a stereoscopic microscope 3 weeks later for detection of viable larvae. Cysts samples from each treatment were also stored for 6 months and 1 year before again exposing them to potato leachings or before opening them for viability determination. In some experiments, treated cysts were placed in steamed soil in which a potato plant was grown, to observe whether females developed on the roots.

EXPERIMENTAL.—*Fumigation of Burlap Bags.*—Exploratory tests were conducted in the 8-cu. ft. drum. A total of 51 tests were made with dosages of 2 to 25 ml. of methyl bromide per cu. ft. of space, including the bags. Exposure periods varied from ½ to 24 hr. The burlap bags were tied into loose bundles of 50 bags each and 2 such bundles placed in the drum. Small nylon bags of cyst material (about 1000 cysts) were placed in various positions in the drum. The fumigant was introduced at the top of the chamber while temperatures were 65° F. or higher. After the desired exposure period, the cysts were removed and placed in petri dishes of filtered potato root leachings. Larvae were counted after 2 weeks at 78° F.

Results indicated that for short (2 hr.) and overnight periods (16 hr.), dosages of 12 ml. and 6 ml. of methyl bromide per cu. ft., respectively, were required for a complete kill. Experiments were repeated several times at these dosages and exposure periods. The cyst material was mixed with both wet and dry soils, and in all tests, no larvae emerged.

Vault fumigation was tested next. The metal lined vault was maintained at 75° F. or higher for the first tests. Twelve bundles, each of 50 loosely tied burlap bags, were placed in the chamber along with the small bags of cyst material buried at different positions. The cysts were removed after the desired treatment periods and placed in potato root leachings for larval counts. Earlier results were confirmed. Experiments also were conducted with cyst material buried in small bags of wet, moist, and dry soil and in triturated potato tubers with similar results. Larval counts showed 27,500 larvae in the untreated material and none in the treated cyst material. Soil samples as large as 10 lb., in doubled-walled paper bags, also were successfully fumigated at these dosages. The tops of the bags were left open to allow the fumigant to penetrate the soil. Here the larval counts were 80,000 for the untreated compared to none in the treated.

The tarpaulin of warp sateen impregnated with ethylcellulose was the next type of enclosure used. The loosely tied bundles were placed on a sheet of glue-coated paper, with the tarpaulin placed over them and sealed at the bottom with a canvas "snake" (canvas tube, 4″ diameter, filled with sand). Results again confirmed earlier tests in that no larvae emerged from any of the treated samples.

Since burlap bags are commercially baled tightly with a press, it was desirable to test commercial bales. Stacks of 300 bags were pressed into bales with a commercial press. Small nylon bags of cyst material were inserted in the center of each stack. Tests were conducted at 50° and 80° F. with dosages of 12 ml. per cu. ft. (46 lbs./1000 cu. ft.) for 2 hr. and 6 ml. per cu. ft. (23 lbs./1000 cu. ft.) for 16 hr. Ten replicate samples of about 500 cysts were included in each bale. After treatment, cysts were placed in leachings and larval counts made. Treatments were again found effective. After weekly counts for 5 weeks no larvae were recovered from samples treated with 23 lb. for 16 hr. In the 46-lb. (2 hr.) treatments, a mean of 0.02 larvae was recovered at 50° F. and 0.088 larvae at 80° F. In each case all larvae recovered were from 1 replication. There were 710 larvae in 1/10 the volume of cyst-leachings mixture of untreated material. These cyst samples were air dried and stored in coin envelopes for 1 year. The replications were thoroughly mixed together and 2 replications of 200 cysts counted and placed in potato root leachings for larval counts to be made at the end of 2 and 4 weeks. No viable larvae emerged from the treated cysts after the end of 4 weeks, compared to 768 in 1/10 volume of the cyst-seepings mixture of untreated check material from the same source as the treated cysts. Opening the cysts from these treatments disclosed no cysts with viable contents. Potato roots growing in pots of steamed soil which had been infested with 0.1 gm. of the treated cyst mixture disclosed no immature females whereas the same amount of untreated cyst material resulted in 81 immature females per gm. of root.

Fumigation of Machinery.—Two types of flexible covers were used in these tests. The first was the heavy fabric coated with vinyl resin. Seventeen small nylon bags of cysts were placed at various positions on a 2-ton stake truck. The truck was rolled upon a vinyl resin coated cover and the experimental cover placed over the truck. A canvas "snake" was used as before to seal the bottom of the cover (Figure 1). The methyl bromide was measured by weighing and was passed through a plastic tube to the top of the truck just behind the cab. No circulation fan was used. In each of the 2 experiments conducted, the dosage was 23 lb. per 1000 cu. ft. of space beneath the cover. After the 16-hr. exposure period, the bags of cysts were removed and placed in potato-root seepings and larval counts made as before. No larvae emerged in any of the 17 replications in each experiment.

The next experiment was conducted with the vinyl resin coated cover over a farm tractor. A dosage of 46 lb. per 1000 cu. ft. was used for a 2-hr exposure. Seventeen bags of cyst material were placed at vari-

FIG. 1. A 2-ton truck being fumigated with methyl bromide confined beneath a vinyl resin impregnated tarpaulin. The methyl bromide is measured by weighing on the scale at the left and enters the enclosure through a ¼-in. plastic tube.

ous points on the tractor and after the fumigation period they were removed and placed in leachings. Larvae present after 1, 2, 3, and 4 weeks were counted. After each reading, the larvae were screened off and fresh leachings added. Only 5 viable larvae were found in the 17 replications which received methyl bromide treatment. From the same number of untreated cysts over 192,000 viable larvae emerged. The opening of 100 cysts from each replication failed to show any viable larvae except in the untreated. Treated cysts, dried and stored for 1 year, when subjected to potato root leachings again for 4 weeks failed to produce viable larvae. A random sampling of 200 of these cysts were opened beneath a stereoscopic microscope but no cysts with viable contents were discovered.

The final experiment was conducted with the polyethylene film covering a ½-ton pick-up truck (Figure 2). Seventeen replicate samples of cyst material

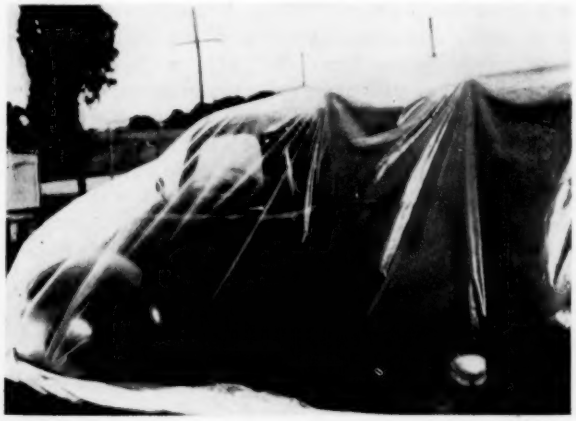

FIG. 2. A pick-up truck covered with a polyethylene film cover. Methyl bromide was introduced into the enclosure through a plastic tube under the cover just behind the truck cab.

were placed at various points on the truck and the methyl bromide used at a dosage of 46 lb. per 1000 cu. ft. for a 2-hr. exposure period. Larval counts showed a mean of 0.094 larvae in the treated samples, all coming in 1 replication. An equal number of untreated cysts produced over 18,000 larvae during the same period. These cyst samples were dried and stored for 1 year, before all replications were mixed together. Two replications of 0.1 gm. of the cyst mixture were placed in pots of steamed soil and a potato seed piece planted in each pot. When white females were evident on the roots of potato plants of check pots, all roots were harvested and counts made of the immature females per gram of root. No immature females were found in the treated material whereas a mean of 81 per gm. of root were disclosed on roots growing in pots infested with same amount of untreated cyst material. Other replicates of 200 cysts were counted and placed in petri dishes of potato root leachings and larval counts made after 2 and 4 weeks. No viable larvae emerged from treated cysts.

DISCUSSION.—Following the discovery of the insecticidal properties of methyl bromide by Le Goupil (3) many uses for this very volatile chemical have been found. Its penetrating ability makes it valuable for fumigation of foodstuffs and graineries. In solving the problem of infestation described, a chemical which readily penetrates baled bags and tiny crevices in machinery is essential. However, the volatility and poisonous nature of this material makes it difficult to use unless the proper facilities are available.

An overnight treatment (16 hr.) at a dosage of 23 lb. per 1000 cu. ft. appeared to have killed all nematodes present. Perhaps this would be a better treatment from the standpoint of cost and convenience than the 2-hr. one at a higher dosage rate. The cost of treatment can be reduced by making sure that the fumigation enclosure is filled with equipment or bags. It also is suggested that the machinery be washed with a stream of water or steamed by the present method before fumigation takes place. This would help remove soil caked beneath wheel fenders and other parts of machinery. Whether methyl bromide will penetrate such hard dried soil is not known since no experiment involved this type of test. It would appear, however, from the results obtained in treating larger samples of soil and ground up tuber tissue that adequate penetration does occur. No damage to machinery receiving these treatments has been observed.

For bag fumigation, the use of flexible covers or a small drum probably would be better than a large vault. Since the vault is stationary, the bags must be brought to a central location which would increase the danger of contamination on vehicles, clothing, etc.

There are precautions which must be observed by anyone using methyl bromide. The choice of the cover is very important. It must be of a material which has been tested and found adequate to retain the gas. Ordinary canvases and waterproofed materials are entirely inadequate. The edges of the cover should be tightly

sealed with a canvas "snake" or wet sand. The site of the fumigation also should be considered. The use of a concrete floor or of some gas-proof material for the botton is recommended. There should be an air space or expansion dome between the top of the material and the cover, and the methyl bromide should be introduced in this space at the top of the material. It is best not to make treatments at temperatures below 40° F. as most covers will crack at these temperatures. The dosage of methyl bromide at low temperatures probably will have to be increased and a volatilizer used since methyl bromide remains a liquid below 40° F. These studies included temperatures down only to 50° F.

If a fumigation chamber is used, it should be of the proper construction, lined with metal and all doors and ports provided with adequate gaskets. An exhaust fan also is important.

Finally, since methyl bromide is an extremely poisonous material and odorless, great care should be exercised in its use. There should always be adequate ventilation in the fumigation area and one should make sure that the fumigation enclosure is adequately ventilated before removing the bags or moving the equipment. The use of an approved gas mask is recommended.

SUMMARY

Fumigation of burlap bags in loosely tied bundles of 50, and in pressed bales of 300, containing cysts of *Heterodera rostochiensis* Wollenweber, resulted in complete eradication at a dosage of 23 lb. methyl bromide per 1000 cu. ft. for 16 hr. Almost equally effective was a treatment of 46 lb. per 1000 cu. ft. for 2 hr. The bags were fumigated in an 8 cu. ft. metal drum in a metal-lined vault, and beneath a warp sateen cover coated with ethylcellulose with equal efficacies. Fumigation of farm machinery at 23 lb./1000 cu. ft. for 16 hr. beneath vinyl resin coated covers and a polyethylene film cover, resulted in eradication of viable cysts placed at several points on the machinery. Treatments were equally effective at temperatures between 50° and 80° F.

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THE PRODUCTION AND ROLE OF ANTIBIOTICS IN SOIL. IV. ACTIDIONE AND CLAVACIN [1]

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The search for new antibiotics has discovered great numbers of soil inhabiting organisms which can produce such materials in laboratory media. Usually, from 20 to 40 per cent of the total number of actinomycetes, bacteria or fungi which are isolated show some inhibitory action. Demonstration that such syntheses of antibiotics also occur in soil would greatly strengthen the hypothesis that these metabolic products play an important part in the antagonisms among the soil microorganisms and influence the composition of the microflora. Furthermore, this information could be of practical interest to the plant pathologist because establishment in the soil of a population of microorganisms antibiotic to the soil-borne pathogens might reduce the incidence of plant disease.

The literature and general background of this problem have been discussed in the 3 previous papers which have dealt with streptomycin, aureomycin, terramycin, and Chloromycetin (chloramphenicol) (2, 7, 9). Of the 4 materials, only chloramphenicol was found in sterilized, unamended soil which was infested with the actinomycete. Three pertinent papers on this subject have appeared since that time. Hessayon (6) has reported that trichothecin is secreted in sterile soil by *Trichothecium roseum*. The researches of Brian and Wright (1) indicated that griseofulvin is partially inactivated in normal soil but not in sterile soil. However, the question of the production of griseofulvin in soil was not answered by these experiments. The studies of Gregory et al (3) showed that antibiotics could be produced by *Penicillium patulum*, *Trichoderma lignorum*, an unidentified actinomycete, and a bacillus when these organisms were grown in sterile soil to which nutrients had been added. Even so, these antibiotics were more or less inactivated in non-sterile soil.

Our current paper reports investigations on 2 antibiotics, a neutral compound, actidione, which is an antifungal agent (12, 13), and an acidic material, clavacin, which inhibits both bacteria and fungi (10). The production of clavacin has been studied intensively by Grossbard (4, 5) and Gregory et al (3). In both investigations the production of clavacin was demonstrated only in sterile soil to which various sources of carbohydrates were added. No reports on the production of actidione in soil have been published.

MATERIALS AND METHODS.—The actinomycete, *Streptomyces griseus*[3] and the fungus, *Aspergillus clavatus*.[3] were used as producers of actidione and clavacin, respectively. *S. griseus* synthesized 75 μg. of actidione per ml. of media in a shake flask and *A. clavatus* 650 μg. of clavacin per ml. in liquid surface culture. *Saccharomyces pastorianus* was used to assay soil extracts for actidione, as well as to determine the effects of the antibiotic in the soil. *Bacillus subtilis* and *Pasteurella avicida* were used for the assay of clavacin. Only *B. subtilis* was used for the study of antagonism in soil. Plate assays for actidione were made with *S. pastorianus* in the manner described by Whiffen (12) and were sensitive to 0.1 μg. per ml. Assays for clavacin were made by the chloramphenicol method of Gottlieb and Siminoff (2), and were sensitive to 20 μg. per ml. Throughout these experiments the heavy, dark, prairie loam soil described in earlier papers was used. For the growth of the microorganisms, this soil was adjusted to 60 per cent moisture-holding capacity. All concentrations of antibiotics in soil are expressed on an air dry weight basis. Crystalline actidione and clavacin were used for preparing standard solutions for studying the inactivation and recovery of antibiotics in soil, and for testing the sensitivity of the various test organisms to the antibiotics. Clavacin standards were prepared both in 10 per cent ethanol and in the same solvent adjusted to pH 6.2 with phosphate buffer; the choice of standard varied with the experiments. Actidione standards were prepared with distilled water. Unless otherwise specified, clavacin was extracted from soil with the alcoholic buffered solution, and actidione with water at a temperature of 70° C. *Aspergillus clavatus* grew well in soil but *S. pastorianus* needed the addition of 1 per cent glucose. Unless indicated otherwise the methods in general are similar to those previously described in other papers of this series (2, 7, 9).

RESULTS.—*Actidione.*—Actidione is not readily adsorbed from aqueous solution by sterile soil. Even after 2 hours of shaking with soil the aqueous solution containing 10 μg. per ml. had not lost activity. The antibiotic does not flocculate illite or bentonite clays, nor does it alter the light-transmitting properties of bentonite suspensions in water. A small amount of adsorption of the antibiotic takes place on both types of clay (1 μg. per mg.), but the amount removed is constant over a wide range of antibiotic concentrations, from 5 to 200 μg. per mg. After the addition of actidione to soil most of the active material could be recovered by a water extraction. At 24° C. water extracted only 22 to 24 per cent of the antibiotic, from concentrations of 5 to 20 μg. per gram of soil. A hot-water procedure was more efficient and gave approximately 90 per cent recovery. This method consists of supersaturating the soil to an excess of 1 or 2 ml. of water, heating the suspension to 80° C. for 10 min., and then centrifuging it. All the antibiotic

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TABLE 1.—*The inhibition of S. pastorianus by the addition of actidione to soil*

Actidione μg./gm. soil	Viable cells × 10^{-3} per gm. of soil
0	31,000
5	520
10	162
15	157
20	68

was free in the soil solution between concentrations of 10 and 50 μg. of actidione per gm. of soil.

The inhibition of *S. pastorianus* by the antibiotic in soil indicates its biologic activity in that environment. The growth of *S. pastorianus* in soil was inhibited at concentrations as low as 5 μg. per gm. of soil but was not entirely suppressed even at 20 μg. per gm. (Table 1). *Pythium debaryanum*, a soil-borne pathogen, which in petri plates is sensitive to 2.5-5.0 μg. per ml. of potato dextrose agar, was prevented from growing in soil by 5 μg. per gm.

S. griseus can produce actidione in sterile soil, but only when suitable nutrients are added (Table 2). No antibiotic was recovered from unamended soil by the warm-water extraction procedure even after 19 days growth. The addition to soil of 0.5 per cent alfalfa or oat straw—which might be considered normal soil fertilization—failed to stimulate the production of actidione. On the other hand, when soybean meal was added at the same rate, actidione could be detected after 19 days. Higher rates of application allowed greater and a more rapid synthesis (Table 2).

The detection of antibiotics in soil depends not only on their production but also on their resistance to microbial degradation. The stability of actidione was therefore tested in both freshly dug normal soil and in autoclaved soil. Both soils were treated with 10 μg. of actidione per gm., stored at 24° C., and then extracted with warm water at various periods. Practically all activity was lost in the normal soil after 11 days while only 70 per cent was lost in the sterile soils during the same period. Another series of experiments was made with air-dried, prairie-loam soil, which had been brought to 60 per cent moisture-holding capacity and incubated for 7 days at 28° C. to allow the microbial population to come to equilibrium before adding the antibiotic. A similar trend was found; extraction of the normal soil at 9 and 11 days yielded only 15 and 8 per cent, respectively, of the original concentration of actidione, while in autoclaved soil the amount of antibiotic at these periods was 47 and 30 per cent. The loss of actidione in sterile soil was surprising, but it might be readily explained on the basis of either insufficient autoclaving or subsequent contamination of the soil. Tests for soil sterility were made in nutrient broth but no indication of bacteria, fungi, or actinomycetes was found. Apparently actidione was not stable even in sterile soil for long periods of time. The presence of microorganisms in such soils, however, markedly hastened the degradation of the antibiotic.

TABLE 2.—*Production of actidione in soil by S. griseus*

Treatment	Yield of actidione in μg./gm. 5 days	19 days
None	0.0	0.0
0.5% oat straw	0.0	0.0
0.5% alfalfa straw	0.0	0.0
0.5% soybean meal	0.0	1.3
1.0% soybean meal	0.9	4.4
3.0% soybean meal	0.8	10.1

Clavacin.—Clavacin is similar to actidione in its relative inertness to the action of soil clay. Flocculation of either the illite or bentonite clays did not occur and only negligible amounts were removed from solution by these colloids. Even whole soil, with its high organic content, did not greatly inactivate this material. As little as 10 μg. of clavacin per gm. of previously sterile soil reduced the growth of *B. subtilis* in such soil, from 11.2 × 10^6 in the controls to 0.5 × 10^6 viable cells per gm. in the treated soils. The population was reduced to 0.6 × 10^5 cells in soils containing 20 μg. per gm. and no viable cells could be detected in soils containing 40 μg. per gm. Despite the relatively good activity of the antibiotic in soil, extraction of this material was difficult (Table 3). Ten per cent ethanol buffered at pH 6.2 with phosphate consistently gave the best results but even then the efficiency was only 30 per cent.

TABLE 3.—*Extraction of clavacin from soil to which 50 μg. per gm. had been added*

Solvent	Per cent extracted
Distilled water	13.0
10% ethanol	14.2
90% ethanol .1N to HCl	20.6
90% ethanol .1N to NaOH	0-10
10% ethanol buffered at 6.2	30.0
95% ethanol	27.0
ethyl ether	11.8
chloroform	0-10

Aspergillus clavatus is extremely antagonistic to *B. subtilis* in otherwise sterile soil, and inhibits the growth of the bacillus in both the amended and unamended soil (Table 4). Within 16 days the number of cells in control soils, containing only *B. subtilis*, had increased from 200 to 11,500,000 per gm., whereas in soils which had been infested with *A. clavatus* 2 weeks prior to *B. subtilis*, the population had risen to only 2000. On further incubation the population of the bacteria continued to increase in the control soils but disappeared entirely from the fungus-infested soil. When tryptone was added to soil to make a concentration of 2.5 per cent, the reproduction of *B. subtilis* was stimulated in both the control and the soils containing *A. clavatus*. Despite this enhanced growth the inhibition due to the fungus was still striking and the

TABLE 4.—*The inhibition of B. subtilis by A. clavatus in soil*

Treatment	Day of sampling	Number of colonies per gm. × 10^{-6} B. subtilis	A. clavatus	pH
I. Unamended soil				
B. subtilis	0	0.0002	.....	6.20
alone	16	11.5	.....	6.23
	31	14.5	.....	6.10
	45	25.4	.....	5.92
B. subtilis	0	0.0002	.....	6.20
plus	16	0.002	30.9	6.22
A. clavatus	31	0	21.8	6.10
	45	0	31.5	6.14
II. Soil amended to 2.5% tryptone				
B. subtilis	0	0.0002	.....	6.20
alone	16	231.0	.....	6.80
	31	1320.0	.....	7.60
	45	1370.0	.....	7.73
B. subtilis	0	0.0002	.....	6.20
plus	16	0.098	110.5	8.05
A. clavatus	31	0.670	159.0	7.91
	45	0.540	172.5	7.90

population in the control flasks was about 2500 times greater than in the mixed cultures.

All attempts to recover clavacin from unamended, sterilized soils that had been infested with *A. clavatus* failed, but the antibiotic was found in 1 of the amended soils. The following materials were mixed with soil, singly at the 1 per cent level: corn stover, oat straw, soybean straw, alfalfa hay, glucose, commercial brown sugar and tryptone. Only the brown sugar stimulated clavacin production; 16 μg. per gm. of soil were detected on the third day but none on the seventh day. Other experiments with the same amendments were tested after 16, 31, and 45 days and no clavacin could be demonstrated in these soils. The efficiency of extraction for this antibiotic is relatively poor, since only 30 per cent of the active material can be removed from soil. In these experiments, at least 16 μg. per gm. of soil would have had to be present to obtain detectable amounts of clavacin. We can, therefore conclude that, if the antibiotic had been produced at all in the unamended soils, its concentration was below that level. The complete inhibition of *B. subtilis* by *A. clavatus* in such soil (Table 4) probably was not due to the production of clavacin since more than 20 μg. of clavacin was necessary for the complete inhibition of the bacillus in soil. Yet smaller amounts of the antibiotic, even though not detected, might have contributed to the complete inhibition of the bacillus.

Data on the stability of clavacin in soil show that it is stable in sterile soil for long periods but that it is inactivated in normal soil. When 50 μg. per gm. were added to sterile soil the recovery of 0, 2, 4 and 7 days, was consistently 15 μg. per gm. or 100 per cent of the extractable material. Normal soil, on the other hand, rapidly reduced the concentration of antibiotic. Fifteen μg. per gm. were extracted on the day of the addition but none could be detected from that time on. Either the antibiotic was completely inactivated or the quantity which remained was too small to be detected.

The response of the microflora in normal soil is striking (Table 5). The clavacin treated soils had a higher general population than the non-treated soils. In addition there apparently was a gradual selection of clavacin resistant cells in the treated soil, since at the end of 7 days there was no significant difference between the number of clavacin resistant cells and the general population. Multiplication of the fungi was greatly stimulated in the presence of clavacin and this entire flora was resistant to 15 μg. of antibiotic per ml. when tested on rose-bengal agar. The number of resistant bacteria also changed within 7 days, from 22 per cent in the control to approximately 90 per cent of the total number of bacteria in those soils which received clavacin. During the same period the number of resistant actinomycetes increased from 19 to 61 per cent.

DISCUSSION.—The supposition that antibiotics are produced in soil and that they are involved in the competitive processes among microorganisms in soil is not supported in the case of actidione and clavacin. Yet both antibiotics would in 1 respect be fit for this part since they are not removed from aqueous solution by normal soil. In this characteristic they resemble chloramphenicol (2) rather than streptomycin (9) since the latter rapidly disappears under similar conditions. The corollary that actidione and clavacin would therefore have inhibitory properties in sterile soil has been experimentally proved; this again is in sharp contrast to the behavior of streptomycin which is in-

TABLE 5.—*Response of the soil microflora to the addition of 59 μg. of clavacin per gm. of soil*

Treatment	Days	Number of viable cells × 10^{-5} per gm. of soil: Bacteria G	Bacteria VR	Actinomycetes G	Actinomycetes VR	Fungi G	Fungi VR	Total G	Total VR[a]
Soil control	0	290.0	7.5	66.7	2.6	1.42	1.21	358.1	11.3
	4	186.6	11.7	76.7	5.8	2.27	2.10	265.6	19.6
	7	125.0	27.0	38.3	7.1	1.53	1.35	164.8	35.5
Soil plus clavacin	0	290.0	7.5	66.7	2.6	1.42	1.21	358.1	11.3
	4	461.7	92.3	38.3	17.2	25.45	8.62	528.5	118.2
	7	123.5	111.2	27.7	16.8	87.50	92.10	238.7	220.1

[a] G = general numbers of organisms.
VR = the number of colonies developing on agar amended with 15 μg. of clavacin per ml.

active under similar conditions. Since soil is not sterile in nature, the activity of a compound must be considered under normal conditions. Normally, soil is the scene of intense metabolic activity of great numbers of microorganisms and under such conditions actidione and clavacin rapidly disappear from soil. This inactivation is not unusual, for comparatively few materials can resist degradation by soil organisms: chloramphenicol, streptomycin, actinomycin, and griseofulvin are all decomposed in normal soil (1, 2, 8, 11).

It is conceivable that, despite the gradual degradation of some antibiotics in soil, these compounds nevertheless might inhibit some of the more sensitive microorganisms before the antibiotics themselves were inactivated or adsorbed on the soil colloids. The more important question is whether such materials are even produced. *S. griseus* and *A. clavatus* have not produced the antibiotics in amounts sufficient to be detected in our experiments with autoclaved unamended field soil. This behavior is in contrast to the action of *Streptomyces venezuelae* (2) and *Trichothecium roseum* (6) which can synthesize antibiotics under these circumstances. The absence of clavacin production in such soils is in agreement with the results of Grossbard who used a different fungus, *Penicillium patulum*, as the antibiotic producer. When *A. clavatus*, and the test organism, *B. subtilis*, were grown together in the soil, no antibiotic formation was evident and any antagonistic effects would have to be attributed to other competitive mechanisms (9). Apparently the nutritive conditions are not suitable for the synthesis of antibiotics in this soil. Addition of soybean meal corrected this condition in *S. griseus* infested soil, but this produces a laboratory medium, not soil. The failure of glucose, and the crop residues to stimulate clavacin production is puzzling, for Grossbard (4, 5) showed that the addition of glucose or wheat straw resulted in its accumulation. This difference could be attributed to a difference in soils or to the fact that we used *A. clavatus* and Grossbard infested soils with *Penicillium patulum*. The transitory occurrence of clavacin in the soils containing brown sugar is not unusual since Gregory et al (3) have also shown that a similar phenomonen occurs with other antibiotics.

Despite the fact that production of some antibiotics has been demonstrated in autoclaved soils, or in soils to which various carbonaceous or proteinaceous materials have been added, the occurrence of specific antibiotics in a normal field soil has not yet been shown.

SUMMARY

Neither actidione nor clavacin were removed from aqueous solution by soil. These antibiotics were not adsorbed by, nor did they flocculate illite or bentonite clays.

Both actidione and clavacin inhibited the growth of sensitive microorganisms in soil.

Actidione was not produced by *S. griseus* either in a sterile unamended soil or when 0.5 per cent oat or alfalfa straw were added to this soil. However, it was produced when soybean meal was added, and the amount synthesized in such soil depended on the concentration of the soybean meal.

Actidione slowly lost some activity in a sterile, black prairie loam soil, whereas clavacin was stable under such conditions. In normal, non-sterile soil, both actidione and clavacin were degraded.

Aspergillus clavatus is antagonistic to *B. subtilis* when sterile soil is infested with both these organisms.

Clavacin could not be found in soils infested with *A. clavatus* even when different carbonaceous materials were added to the substrate.

The presence of clavacin in soil caused the rise of a resistant microflora in the soil.

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REACTION OF BRASSICA SEEDLINGS TO BLACKROT [1]

Douglas C. Bain

Seed treatment with hot water, along with rotation and sanitation, is recognized as the most effective method of control of blackrot of cabbage. Despite the effectiveness of this control measure, it was considered desirable to explore the possibility of field resistance in closely related varieties of *Brassica oleracea* and other species of Brassica. This paper presents results obtained during a study of the seedling phase of the problem.

Review of Literature.—There are few publications concerning the reaction of cabbage varieties and related plants to blackrot. Relative to cabbage, Russell (6) stated "there is but little difference in susceptibility, all varieties readily yielding to the disease, if the causal organism is once present." However, among closely related plants he "provisionally" rated cauliflower as the most susceptible, cabbage as "readily affected," broccoli, kohl rabi and Brussels sprouts as "quite susceptible," and collards as least susceptible of the group. Turnip, radish, rutabaga, and "species of this character" were considered as "only slightly susceptible." Edwards (5) concluded from results of inoculation tests, as well as from varietal observations, that the Houser cabbage "is practically immune to blackrot under field conditions." He also pointed out that this variety had been advertised as "proof against blackrot." Bach and Taubenhaus (1), during an epiphytotic of blackrot in 1928-29, ranked Glory of Enkhuizen as the most severely affected, followed in descending order by Copenhagen Market, Flat Dutch, Wakefield, All Head Early, Mammoth Red Rock, and Savoy. Loss among the latter named varieties was small as compared with the first two. Heavy losses of cauliflower and broccoli were also reported, thus indicating a high degree of susceptibility.

Clayton (3) stated that there was no resistance in strains of the Snowball-Erfurt variety of cauliflower but that vigorous coarser types such as Henderson-Erfurt were better able to withstand the disease than certain highly selected strains. He selected strains from Snowball-Erfurt that possessed more resistance than the original variety but this was not considered of a high degree. Selections from crosses with blackrot "tolerant" collard backcrossed on cauliflower were as resistant to the disease as the parent collard. Clayton suggested there was a good possibility of obtaining blackrot resistant cauliflower from those selections.

Thung (7) reported that of several varieties of cabbage tested in Java, Late Giant, Early Drumhead, Sugarloaf and Yellows Resistant showed more field resistance to blackrot than Earliest of All and Yates Utility. Glory of Enkhuizen and 16 other varieties (not named in the abstract) were very susceptible. This substantiates, in part, the observations of Bach and Taubenhaus. Eddins (4) reported that in inoculation tests with 67 strains of cabbage, all plants (10 per strain) became infected but that blackrot developed more rapidly in early maturing varieties than in late ones. He rated as resistant strains of Red Acre, Penn State Ballhead, All Head Select, Wisconsin Bugner, Florida Danish and Savoy Perfection Drumhead. In a recent report on seedling tests with cabbage, the writer (2) rated as resistant Premium Late Flat Dutch, Succession, Red Acre, Marion Market, Wisconsin Ballhead, and Early Round Dutch. Other varieties, including Glory of Enkhuizen and Copenhagen Market, were considered susceptible. It was pointed out, however, that these ratings were based on the general appearance of a large number of seedlings in pots at a particular stage of development of the disease. Attention was called to the fact that among diseased seedlings of different varieties there were usually several plants that remained healthy. It was suggested that these plants might possess a high degree of resistance to blackrot.

Methods and Materials.—The methods used in the major portion of this work were essentially the same as those employed previously (2). Seed were soaked about 24 hours in a concentrated liquid culture of *Xanthomonas campestris* (grown in 400 ml. solution for 4 days), incubated on filter paper in Petri dishes until germination and then potted with the paper. Beginning with the earliest appearance of blackrot on cotyledons or leaves, diseased plants were rogued and counted frequently throughout a period of 2 or more weeks. When it appeared that no more (or very few more) plants would become diseased, the remaining healthy ones were counted and the percentages of blackrot seedlings determined. In many instances, a few of the remaining healthy plants were repotted and kept for seed production.

Results of Cabbage Tests.—Numerous tests were made with over 75 commercial varieties and strains of cabbage. In general, results were much the same in all tests. Some varieties could be arbitrarily classed as susceptible or resistant, depending upon the percentage of blackrot seedlings. Most of the varieties were fairly consistent in the percentage of diseased seedlings in the different tests although there was a reasonable variation from an exact figure. Results of 1 series of tests are in Table 1. Plants which had disease symptoms in true leaves only were counted over a period of about 5 weeks. The average percentages of blackrot plants varied from 13.1 (Jersey Wakefield) to 74.6 (Mammoth Red Rock). When only a very few seed germinated the percentage of diseased seedlings was very low despite a high percentage when germination was high. Examples of this are Allhead Select

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TABLE 1.—*Cabbage varieties. Percentages of seedlings that developed blackrot in true leaves. Plants 43 days old when final notes were taken. Few varieties in 1 series of 3 tests.*

Variety	Total plants tested	Average per cent blackrot
Jersey Wakefield	272	13.1
Allhead Select	205	14.3
Stein's Flat Dutch	365	19.6
Wisconsin All Seasons	252	20.9
Savoy Chieftain	281	24.4
Wisconsin Hollander	176	26.3
Late Flat Dutch	313	27.0
Charleston Wakefield	242	32.6
Huguenot	247	38.8
Stein's Early Flat Dutch	165	50.8
Slow Bolting Flat Dutch	116	57.4
Resistant Glory	197	62.0
Early Glory	187	65.8
Globe	195	65.8
Reed's Ballhead	109	71.7
Mammoth Red Rock	167	74.6

TABLE 2.—*Foreign and domestic cabbage varieties and selections. Percentages of blackrot seedlings.*

Variety	Number tests	Total plants tested	Average per cent blackrot
Resistant Detroit	2	133	96.5
Huguenot selection 1	2	22	0.0
Extra Early Masago	2	242	48.4
Tri Season	2	241	45.8
Early Masago	2	320	18.2
Early Fuji	2	199	5.0
Charleston Wakefield	3	439	75.6
do selection	3	180	32.8
Huguenot	3	680	89.6
Huguenot selection 2	3	202	4.6
Round Dutch selection	3	76	15.1
P.E.I. #163489	3	248	89.1
P.E.I. #169052	3	299	89.0
P.E.I. #163488	3	128	53.7
Houser	3	17	4.8
Succession	4	405	40.4

and Slow Bolting Flat Dutch. Germination tests with seed of susceptible and resistant varieties soaked in inoculum and in water as checks indicated that low germination was not associated with susceptibility. Although Glory of Enkhuizen and Copenhagen Market were not used in this series of tests, results in others would put them in the susceptible class—thus agreeing in part with the observations of Bach and Taubenhaus (1) and Thung (7). In other tests, the reaction of strains of Red Acre and All Head Select was considered as resistant but that of Penn State Ballhead and Savoy Perfection Drumhead was susceptible—which agrees in part with the report of Eddins (4).

On the basis of ratings in these and other tests, 2 resistant varieties (Wisconsin All Seasons and Succession) and 2 susceptible ones (Resistant Detroit and Copenhagen Market) were selected for a series of 12 tests in order to get a clearer picture of the reaction of seedlings to blackrot. Only 1 count (12 days after emergence) was made of plants with symptoms on either the cotyledons or leaves or both in this series. Between 1500 and 2000 plants of each variety were used in the tests. The reaction of resistant (low percentage of blackrot) and susceptible (high percentage of blackrot) varieties in the seedling stage showed up clearly. Except in a few instances, the percentages of blackrot seedlings were fairly consistent. Seventy-six and 68 per cent of the seedlings of Resistant Detroit and Copenhagen Market, respectively, developed blackrot but only 29 and 20 per cent of Wisconsin All Seasons and Succession became diseased.

Of particular interest were results obtained in a series of tests which included not only foreign varieties but also selfed selections from previous tests as well as checks from commercial seed. Counts of plants with diseased cotyledons or true leaves were made over a period of about 3 weeks. There were striking differences in reaction to blackrot among the varieties and selections (Table 2). In general, the P.E.I. group tended to have considerably more varieties in the high percentage of blackrot group than our domestic varieties when compared with other tests. The Japanese varieties had a surprisingly low blackrot percentage, especially Early Fuji and Early Masago, as compared with the resistant domestic variety Succession. The variety Houser had a low percentage of blackrot but so few seeds germinated the results were not considered significant.

TABLE 3.—*Rate of development and percentage of blackrot in cabbage varieties and selections. Twenty plants per variety or selection.*

Variety	Date and number diseased plants 11/23	11/24	11/26	11/27	11/28	12/3	12/6	Per cent blackrot
Charleston Wakefield[a]	5	10	14	18	19	20	20	100.0
do selection[c]	0	0	0	5	6	15	19	95.0
Huguenot[a]	4	10	13	19	19	19	20	100.0
Huguenot[b]	3	6	9	14	16	19	20	100.0
do selection[c]	0	0	0	5	5	8	13	65.0
P.E.I. #163488[b]	2	8	9	15	16	17	18	90.0
Early Fuji[b]	0	0	0	5	8	12	13	65.0
Succession[b]	2	4	4	10	11	14	15	75.0

[a] Plants from noninoculated commercial seed—33 days old.
[b] Healthy plants from inoculated seed—50 days old.
[c] F_1 blackrot-free selections from previous seed inoculations and these, healthy, plants from inoculated seed—50 days old.

Even more interesting were results obtained with selfed selections from blackrot-free seedlings tested in 1950. Seed from commercial lots were used as checks against the selections out of Charleston Wakefield and Huguenot. It will be noted in Table 2 that the percentage of blackrot seedlings of the selections was considerably lower than that of the checks. The selection out of Round Dutch also had a low percentage of diseased seedlings. These results strongly indicate the presence of a factor, or factors, for resistance to blackrot in cabbage seedlings and that the technique employed for testing resistance and susceptibility is reliable.

As a further check on the possibility of blackrot resistance, surviving healthy plants of Charleston Wakefield, Huguenot, the selections, Succession, P.E.I. #163488 and Early Fuji from these tests, as well as plants from noninoculated commercial seed of the first 2 varieties, were inoculated twice in 2 days by stem injection with a needle. The plants from seed inoculations were 50 days old and those from noninoculated seed were 33 days old. Notes were taken over a period of 16 days following the last inoculation. Symptoms of blackrot did not appear in plants of the selections or Early Fuji until 7 days after inoculation (Table 3), but those of the other entries showed very early symptoms (wilting in 1 or more leaves) in 4 days. The rates at which plants of the different entries showed symptoms can be divided into 4 categories. Plants from noninoculated commercial seed of Huguenot and Charleston Wakefield developed blackrot the earliest; those from inoculated seed of P.E.I. #163488 and Huguenot were a few days later; plants from inoculated seed of Succession were third, while those from Early Fuji and the selections out of Huguenot and Charleston Wakefield were last. More plants from the latter selection developed blackrot than either Huguenot selection or Early Fuji. The only plants killed by blackrot in the test were 3 from noninoculated seed of Huguenot. In the early stages of disease development in the selections and Early Fuji, there was an apparent temporary recovery from wilting; however, the infected areas eventually became necrotic. There were a few plants among the entries that did not become diseased.

RESULTS OF TESTS WITH BRASSICAS OTHER THAN CABBAGE.—Two tests were made on a large number of different varieties of *B. oleracea*, excluding cabbage, as well as other species of the genus. Results of these tests are in Table 4, but for brevity only the entries (except cauliflower) having the highest and lowest percentages of blackrot seedlings are listed.

By far the lowest (3.6) average percentage of diseased seedlings occurred in the *B. nigra* group while the highest (99.3) was in the 10 domestic commercial strains or varieties of cauliflower. Rape, commercial varieties of mustards, *Brassica* spp. (mostly mustards) and varieties of turnip were in the low blackrot bracket. Rutabaga, collard, kale (except Siberian), Brussels sprouts, broccoli and kohl rabi were in the high blackrot group. Thus, those plants most closely related to cabbage could be considered as susceptible (cauliflower especially so) to blackrot while the other species were tolerant to highly resistant.

Of particular interest was the rather extreme difference between the reaction of domestic and foreign (P.E.I. accessions) varieties of cauliflower—the latter had an average of 22.5 per cent diseased seedlings while that of the domestic varieties was 99.3. Even though the 2 groups were not tested at the same time results with other varieties indicated that this difference in reaction of the cauliflowers was not due entirely to environmental or pathogenicity factors.

Of the kale varieties, Dwarf Siberian and 1 strain of Siberian had the lowest percentages of blackrot seedlings (18.7 and 7.7 respectively), but a variety listed as Siberian Improved had an average of 82 per cent diseased seedlings and other varieties were almost equally susceptible or even more so.

Seedlings of P.E.I. accession No. 180261, listed as

TABLE 4.—*Species of Brassica. Percentage of seedlings that developed blackrot on cotyledons or leaves or both. Results of 2 tests.*

Species or Variety	Total plants	Average per cent blackrot	Per cent blackrot for all varieties
Turnip (19)			
Purple Top	506	80.8	
P.E.I. #163494	405+	1.2	32.9
Mustard (11)			
Fla. Broadleaf	506+	1.0	
Tendergreen	371	65.0	18.8
B. nigra (8)			
P.E.I. #169057	268+	0.8	
P.E.I. #180416	434+	7.7	3.6
Rutabaga (10)			
P.E.I. #164841	179	81.1	
P.E.I. #180261	268	0.0	58.4
Cauliflower (18)			
10 Com. Vars. Av.	2459	99.3	99.3
P.E.I. #180260	228	22.4	
P.E.I. #181719	226	27.9	
P.E.I. #183212	30	17.3	22.5
Collard (4)			
Georgia	234	79.4	
La. Sweet	411	49.2	63.9
Kale (9)			
Dwarf Siberian	233	7.7	
Dwarf Green Curled Scotch	279	97.3	67.7
Brussels Sprouts (3)			
Half Dwarf Improved	240	56.4	
Long Island Improved	424	76.5	67.7
Kohl Rabi (8)			
Early White Vienna	477	97.2	
P.E.I. #188611	44	19.2	75.6
B. napus (10)			
P.E.I. #169075	523	22.8	
P.E.I. #169085	190	1.1	12.5
B. species (10)			
P.E.I. #179649	173	0.0	
P.E.I. #179650	240	77.6	21.3

NOTE: Parenthetical figures following name indicate the number of entries.

B. campestris and therefore presumably rutabaga, and No. 179649 (*Brassica sp.*) developed no blackrot at all. This has happened in tests with cabbage but was associated with low germination—i.e., when only a few seeds out of an estimated 225 germinated often none of the seedlings would become diseased; however, there were well over 150 seedlings of the P.E.I. numbers.

Discussion.—Results of a large number of tests involving more than 200,000 seedlings showed that there were always (with few exceptions in cauliflower) plants which did not become diseased as a result of seed inoculation. These healthy seedlings either escaped infection or they possessed a factor or factors for resistance to blackrot under the conditions to which they were subjected. Since all the seeds in any variety in any test were subjected to the same amount of inoculum for the same period of time under equal conditions (and likewise incubated and germinated), it seems reasonable to assume infection would occur in all of them if they were equally susceptible. Results of 1 test with cabbage, however, wherein healthy seedlings from a seed-inoculation test were inoculated by stem injection, showed that in some varieties (Huguenot and Charleston Wakefield) all were susceptible but in other varieties (Succession and Early Fuji) some of the healthy seedlings were highly resistant—i.e., some plants did not develop blackrot after seed inoculation and even stem injection of inoculum later. Not only were there a few plants in the 2 resistant varieties that remained healthy but also the rate of disease development was slower than that of susceptible varieties. This substantiates results of seed-inoculation tests wherein Huguenot and Charleston Wakefield usually had a high percentage of blackrot seedlings while Succession and Early Fuji had a low percentage of diseased plants. Thus, a few seedlings appear to escape seed infection but most of these apparently are not so highly susceptible as ones that become diseased following seed inoculation.

The degree of seedling infection was not given particular attention in any of the tests; however, observations led to the conclusion that there are degrees of severity of the disease between seedlings of botanical varieties and species and even between commercial varieties. These degrees could be described as follows: (1) yellowing of cotyledons with slight necrosis, (2) rather slow but progressive necrosis in cotyledons, and (3) rapid necrosis and often eventual killing of seedlings. The first is characteristic of turnips and mustards and the last of cauliflower; the other groups are, in general, intermediate. Some of the P.E.I. cabbages, however, were as severely affected as cauliflower.

In the light of work herein reported, it would appear that there is a factor or factors for resistance to blackrot in cabbage seedlings, and possibly in related plants, and that this resistance can be selected out of certain commercial varieties.

SUMMARY

The reaction of seedlings of a number of varieties and strains of Brassica to blackrot was obtained by determining the percentage of diseased plants from inoculated seed.

Turnip, mustard, *B. nigra*, *B. napus* and *Brassica spp.* (mostly mustards) were in the low (less than 33) percentage blackrot group and cauliflower, broccoli, rutabaga, collard, kale, Brussels sprouts and kohl rabi were in the high (more than 55) percentage blackrot group while cabbage was intermediate (41). Varieties and strains within these groups varied from high to low percentage of blackrot.

Selfed selections from surviving healthy seedlings of cabbage had a marked reduction in percentage of diseased seedlings as compared with checks from commercial seed.

The possibility of selection for resistance to blackrot is discussed.

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FOREST HUMUS QUALITY AND LIGHT CONDITIONS AS FACTORS INFLUENCING DAMPING-OFF [1]

Olli Vaartaja

In nurseries and greenhouses damping-off of tree seedlings is commonly caused by virulent parasites such as *Rhizoctonia solani* or *Pythium* spp. These fungi can be inhibited or parasitized by *Aspergillus niger, Botrytis cinerea, Penicillium* spp., *Gliocladium* sp. (22) and *Trichoderma* sp. (5,22 etc.), which are common in forest soils (16). The natural forest soil with its rich fungus flora is probably a substratum that can be said to be "biologically well buffered". But in greenhouses and nurseries the biological equilibrium is often disturbed in many ways, resulting in conditions favorable to the outbreak of epidemics of virulent pathogens.

Another contrast between natural and artificial seedbeds is found in their microclimates. The crowns of trees and ground vegetation, as well as litter, reduce the radiation which reaches germinating seeds and seedlings in forest stands. In nurseries the seedbeds are usually more exposed.

The object of this study was to determine the influence of forest fungus flora and humus on damping-off under several temperature and light conditions. In the principal experiments seeds and seedlings were not directly inoculated but were exposed to the whole flora of the humus. In other experiments the pathogenicity of certain isolated fungi was demonstrated in pure cultures, and the germination of the experimental seed tested under different conditions in the absence of fungi.

SEEDBED EXPERIMENTS.—*Methods.*—For preparing experimental seedbeds, forest humus and surface soil samples were collected in winter and stored at freezing temperatures until used in the laboratory. They were from natural sites in southern Finland representing different forest types in Cajander's sense (2). Only samples from dense, mature spruce and pine stands were used.

Seedbeds were of 2 types, the one of humus whose surface was retained in natural conditions as much as possible, and the other a mixture of humus and sand. "Humus seedbeds" were put into galvanized iron flats of 2 × 20 × 30 inches. "Sand-humus seedbeds" were prepared in clay pots using a mixture of about ¾ sand and about ¼ humus. The sand was a fine-textured subsoil from a pine stand.

In the laboratory the pots were kept under an illumination intensity equivalent to that under the canopy of a very dense spruce stand near Helsinki: the maximum illumination used was about 400 foot-candles varying from 1 to 10 per cent of that in the open. In the humus seedbeds corresponding values were 200 to 300 foot-candles or 3 to 10 per cent. Outside experiments were unshaded or only moderately (50 per cent) shaded; maximum illumination was about 10,000 and 5,000 foot-candles respectively.

In pot experiments in the laboratory the duration of illumination was also controlled. It was regulated by fluorescent lamps or black curtains. Two durations were used: continuous illumination (only 3 foot-candles at night) or 10 hours of daily illumination. Outside experiments were conducted from June to August, 1951. The experiments with humus seedbeds were conducted from January to February, 1951.

Abundant moisture was provided in all experiments. Temperatures in laboratory experiments varied a few degrees daily; extremes are shown in the tables which follow.

In general 50 seeds of each species (25 of *Larix*) were sown in individual experiments. Amounts of germination and damping-off were determined weekly. At that time dead seedlings were removed. Germination was considered to have occurred if the radicle tip had clearly elongated beyond the seed coat.

The mortality of seeds (M_1) and seedlings (M_2) and the final survival (S) were calculated as percentages according to the following formulas:

$M_1 = 100(V - G)/V$, where G is actual germination, and V is number of "viable" seeds

$M_2 = 100\ D/G$, where D is number of dead seedling

$S = 100(G - D)/V$

The number of "viable" seeds was assumed to be the same as the highest average germination in the replicated tests made under various light and temperature conditions on sterile sand.

Pot experiments in the laboratory.—The *Pinus silvestris* L. seed germinated promptly and showed low mortality. On a seedbed with humus from the *Vaccinium* type all viable seeds germinated. Exceptionally high mortalities, 24 per cent, were encountered in 2 samples, the one from the *Oxalis-Maianthemum* type and the other from the *Oxalis-Myrtillus* type.

The *seedling* mortality of pine varied widely (Table 1). The difference between seedbeds representing types of high (OMaT + OMT) and intermediate fertility (MT + VT) was great and statistically significant (50 ± 8.1 per cent).

According to Aaltonen (2) the percentages of total nitrogen in the humus of different forest types are: CT 1.50, VT 1.67, MT 1.80, OMT 2.23, and OMaT 2.80. The amount of NO_3-nitrogen, NH_4-nitrogen, potassium and calcium as well as pH in the soils and timber yield increase in the same direction (2,16). The properties of different humus materials as seedbeds and as sources of plant nutrients seem to be quite opposite. Possibly the high mortalities on OMaT and OMT were caused by the large amount of nitrogen,

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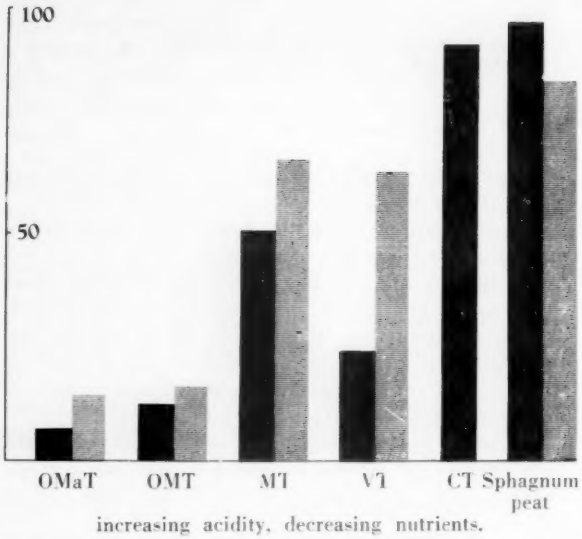

FIG. 1. Final survival (percentage of optimum germination) of pine as influenced by seedbed quality and different periods of illumination: low light intensity. *solid* = in "short days"; *shaded* = in continuous illumination. Number of samples: OMaT 8; OMT 5; MT 6; VT 2; CT 1; peat 2.

known to decrease disease resistance of plants in general.

The final survival of pine is illustrated in Figure 1. There was a tendency for survival to be a little higher under continuous than under 10 hours daily illumination. A contrary case where the samples were too few may have been accidental.

This tendency for survival to be influenced by daily illumination duration was great in the experiments with *Betula verrucosa* Ehrh. (Table 2). Seed mortality was 21 ± 6.1 and seedling mortality 24 ± 8.3 per cent less in continuous than in 10 hours daily illumination. Differences were also great in experiments with *B. pubescens* Ehrh. (Table 3).

The general tendency shown by other species, in Table 3, is similar to that indicated in Tables 1 and 2, although the numbers of samples were smaller. However, *Picea abies* (L.) Karst. and *Larix sibirica* Led. showed larger seed mortalities than *Pinus silvestris*. This difference was probably due to different qualities of seeds, at least the seed of *Picea abies* being of poor quality (2 years old).

TABLE 1.—*Mortality of Pinus silvestris seedlings as influenced by seedbed quality (seedbeds of humus-sand mixtures, light intensity low, temperature 18–25° C.)*

Humus mixture from	Samples No.	Mortality, per cent (Mean ± standard error)
Sphagnum peat	2	7
CT (*Calluna* type)	1	7
VT + MT (*Vaccinium* and *Myrtillus* types)	10	37 ± 6.5
OMT + OMaT (*Oxalis-Myrtillus* and *Oxalis-Maianthemum* types)	14	87 ± 4.8

Germination, particularly that of birch, was low even in special experiments on sterile sand seedbeds when temperature was about 20°C. and daily illumination amounted to 10 hours of rather low intensity. When these sterile seedbeds were exposed to stronger and continuous illumination, germination, which had been inhibited, was again resumed. However, in ordinary experiments where seeds were exposed to the influence of fungi, similar increases in strength and duration of illumination resulted in no new seedlings; the seeds were found to be rotten. Short daily duration of illumination had definite, but perhaps only indirect, effects on seed mortality by delaying germination and thus increasing the length of exposure of the seeds to parasites.

Diseased seedlings showed girdling at the stems or dying-back from the tips. Death of the seedlings was evidently caused by fungi, not by shading. In sterile conditions seedlings survived even under much weaker illumination intensity; growth however, was slow. Under short daily illumination seedling growth was inhibited in a characteristic way (20).

Pot experiments outside.—In experiments performed outside, humus samples were mainly from the same forests as those in laboratory experiments. Pots representing 16 samples were in full sun light while 18 were moderately shaded (50 per cent). Results in both circumstances were in clear contrast to those obtained under lower light illumination intensities: *experiments conducted outdoors showed no marked seed or seedling mortality.*

TABLE 2.—*Mortality of Betula verrucosa seed and juvenile seedlings as influenced by light and seedbed conditions (seedbeds of humus-sand mixtures, light intensity low, temperature 18–25° C.)*

Humus mixture from	Samples No.	Illumination: Mortality of seeds per cent		Illumination: Mortality of seedlings per cent	
		10 hrs./day	continuous	10 hrs./day	continuous
Sphagnum peat	1	100	45		20
Vaccinium type	2	51	20	100	39
Myrtillus type	2	39	29	100	61
Oxalis-Myrtillus type	2	41	26	100	100
Oxalis-Maianthemum type	4	65	33	100	91
Mean ± standard error	11	47 ± 5.1	25 ± 3.5	100 ± 0	76 ± 8.3

TABLE 3.—*Mortality of seed and juvenile seedlings of certain species as influenced by light and seedbed conditions (seedbeds of humus-sand mixtures, light intensity low, temperature 18–25° C.)*

Species	Sphagnum peat	CT	MT	OMaT	Sphagnum peat	CT	MT	OMaT
	Mortality of seed per cent				Mortality of seedlings per cent			
Picea abies	10	0	8	51	2	9	11	78
Larix sibirica	22	40	20	39	7	24	57	92
Betula pubescens "short days"	100	—	—	100		—	—	—
Betula pubescens, "long days"	84	—	—	90	18	—	—	100

Even in the 6 pots containing humus from the OMaT seedling mortality was only 0-10 per cent, and this was partly caused by insects. If there were differences in mortality owing to fungi, they were evidently small. Results of these experiments as compared with those in the laboratory suggest that such factors as high illumination intensity, or perhaps great temperature variations, can have an inhibiting effect on damping-off, at least on certain types of the disease.

Humus seedbeds.—The tendency found on natural, unbroken humus seedbeds (Table 4) is clear, although there were incidental irregularities especially where germination was low. On fertile humus (OMaT) mortality was highest. Thus, the tendency confirms the findings of pot experiments. The inhibiting effect of short daily illumination on the germination of birch was again encountered and in more pronounced degree. This is probably attributable to the lower temperatures. When, after the ordinary experiments, temperature and illumination were improved, only a few new seedlings appeared. Most ungerminated seeds were found to be rotten.

The mortality of seedlings was caused chiefly by damping-off fungi except in some cases where a snail ate a few seedlings.

Some of the pine seeds were injured by a small footless insect larva. This kind of injury was reduced, however, by removing all insects and snails that were detected. Many small animals were found on seedbeds but these never appeared to cause injury to seeds and seedlings. Aracnoids were often found to eat *Penicillium* and *Trichoderma* on the surface of seedbeds.

TABLE 4.—*Mortality of Finnish tree seed and juvenile seedlings as influenced by seedbed conditions. (Unbroken humus seedbeds in flats. Short days and low light intensity. Temperature 10–15° C.)*

Species	OMaT	MT	CT	OMaT	MT	CT
	Mortality of seed per cent			Mortality of seedlings per cent		
Pinus silvestris	8	1	4	100	[16]	10
Picea abies	59	18	[41][a]	100	0	[22]
Larix sibirica	[71]	[34]	[41]	[91]	[33]	0
Betula verrucosa	100	92	96	[?]	[100]	[?]
Betula pubescens	99	99	98	[?]	[?]	[?]

[a] Values in brackets = if the extreme values of 4 replications differed more than 10 per cent.

STUDIES ON THE PATHOGENS.—Diseased tissue was taken from dying experimental seedlings, surface sterilized by flame, sodium hypochlorite or alcohol, and placed on malt agar. Outgrowing mycelia, except *Penicillium* spp., *Rhizopus* spp., some species of *Mucor* and *Trichoderma* sp., were isolated and cultured on malt agar.

Using the standard technique (9) sterile seedlings were inoculated with some of the isolated fungi. The medium consisted of: 1 liter of distilled water, 15 gms. Bacto agar, 0.5 gm. KH_2PO_4, 0.05 gm. $CaCl_2$, 0.025 gm. NaCl, 0.15 gm. $Mg\ SO_4 + 7\ H_2O$, 0.2 gm. $(NH_4)_2\ SO_4$, and 1 ml. of a minor elements solution (15). Each culture (usually in a 500 ml. Erlenmeyer flask) consisted of 4 to 11 seedlings.

The inoculation was done, when possible, by sprinkling a spore suspension on the seedling, or by putting a small piece of agar bearing mycelium at the base of seedling. In the controls distilled water was used instead of the spore suspension. Each treatment was replicated 3 times.

Isolations and pathogenicity tests.—At least 17 possibly parasitic fungus species were obtained from 64 successful isolations. Among those 17 species, *Papulospora* sp., *Mucor ramannianus* Möller[2], two *Fusarium* spp. and *Botrytis* (*cinerea* Pers?) were most common. Also *Trichoderma* sp., *Hormodendron* sp., *Gliocladium* sp., *Stemphylium ilicis* Tengw., *Stemphylium* sp., *Alternaria tenuis* auct., (?) *Monilia* sp., *Fusarium* spp., *Dematium* (*pullulans* de Bary ?) and *Rhizoctonia* spp. were isolated. Species of the last mentioned genera were not *Rh. solani*. No *Pythium* sp. was isolated.

[2] *M. ramannianus* was identified by V. M. Cutter, Jr., Yale University.

TABLE 5.—*Mortality of Pinus silvestris seedlings as exposed to isolated fungi under various light conditions.*

Illumination intensity f.c.	Illumination duration hrs./day	Temperature °C	Fungus isolate 374	382	384	No. Control
			Mortality, per cent			
7	continuous	20	94	25	100	25
60	continuous	20	41	22	22	6
450	continuous	20	0	0	10	0
60	continuous	15	86	31	21	5
60	18	15	100	21	44	22
60	12	15	100	35	58	5
60	8	15	100	39	94	4

In pathogenicity tests, mostly carried out in continuous illumination of 50-100 foot-candles at room temperature, 8 of 9 tested fungus isolates showed strong or moderate pathogenicity on pine or birch seedlings. *Mucor rammanianus* was the only fungus tested not capable of killing birch seedlings, at least not rapidly. This fungus killed some of the pine seedlings but those only with difficulty, usually after a very profuse mycelium was formed.

The effect of temperature and light conditions.—Three fungus species were used in experiments where pine seedlings were exposed to various illumination conditions and temperatures. The sources of illumination were fluorescent tubes of daylight type.

When inoculated, the seedlings were developing cotyledons. Most damping-off occurred after 2-4 weeks. Little more damping-off occurred after 5 weeks except in experiments at 7°C with the isolate 374 (*Botrytis*) which finally killed all seedlings. The pathogenicity of this fungus was very strong at low temperatures. This may result from the fact that optimum growth and enzyme production of *Botrytis* (*cinerea*) occurs at low temperature, as established by Gäuman and Näf (3). On the other hand the isolate 384 (*Gliocladium* sp.) showed highest pathogenicity at 30°C. Only 15 and 20°C from 4 temperatures tested allowed clear pathogenicity of the isolate 382 (*Mucor ramannianus*) during 5 weeks.

Table 5 illustrates the effect of light conditions on the pathogenicities of these isolates.

In general, replications showed about the same results. In some cases a difference was found, probably caused by the increased virulence of an isolate if a seedling happened to have a low resistance and after a large mycelium was formed on it. A number of re-isolations regularly produced the inoculation fungi.

These experiments indicated that even at very different temperatures at least some of the isolates were capable of strong pathogenicity. The virulences of all 3 tested isolates were evidently lower, or the resistance of seedlings higher, if the cultures were well lighted. The differences under various durations of daily illumination were not great but all were in the same direction and in accordance with the results of the humus experiments.

Insufficient surface sterilization of seeds caused many contaminations of cultures. Heavier sterilization, however, caused disturbance in germination and could not be applied. If contamination appeared before inoculation, the cultures were kept under illumination of 7 or 60 foot-candles and the pathogenicity of the fungus was studied. Evident pathogenicity was shown by 5 species (*Alternaria* sp., *Fusarium* sp., *Hormodendron* sp., *Rhizoctonia* sp., etc.). Some suggestion for pathogenicity was shown by 7 other species (*Aspergillus* sp., *Neurospora* sp., *Dematium* sp., *Hormodendron* sp., *Fusarium* sp., etc.). Only few contaminations seemed to be obligate saprophytes. These observations support the former results indicating that many fungi can be pathogenic under low light intensities.

Ecological relationships.—Although difficult to observe, damping-off has been found in many places in forest stands (4, 5, 6, 13, 14, 17 etc.). After following closely the development of experimental seedlings in different plots in northern Idaho, Haig (4) found damping-off incidence varying from 6 to 68 per cent. Under dense canopies fungi or drought caused most of the seedling loss. L. F. Smith's experiments (13) in New England showed similar results. D. M. Smith (14) found in Connecticut that damping-off incidence was low on open plots but high on plots with shade: under heavy pine slash the percentage of seedlings killed by fungi was 83; under hardwood brush it was 24, but on open places on lichen and mineral soil only 2.5 and 1.4. Similarly, in Finland on an open burnt heath, mostly lichen-covered, no damping-off occurred (19). But considerable damping-off was found in Finland under the shade of ground vegetation on somewhat more fertile unburnt seedbeds of humus and moss (6). In pot cultures in Connecticut the mortality of seedlings was high on undisturbed humus but low on burnt-over humus and on denuded sand (8). In pot cultures in Wisconsin damping-off was common unless poor sandy soil was used (23).

Because results of these field and pot experiments performed in various conditions with different tree species agree with those of this study, the following conclusions may apply rather generally: (1) Damping-off occurs on natural forest humus but, (2) is rare on poor sites and (3) is often encouraged by shade. Damping-off occurs widely in forests because it can be caused by a great number of fungi, some of which (e.g. *Botrytis cinerea* and *Mucor ramannianus*) are common in forest soils. These fungi may infect seedlings that are not in proximity to diseased seedlings. Probably the high concentration of seedlings usual in experiments and in artificial seedings encourages damping-off but it is not an essential pre-disposing factor.

The effect of shade on damping-off caused by numerous soil fungi was similar to that found in experiments with the following fungi and hosts: *Fusarium* spp. on Scotch and red pine seedlings (18), *Botrytis cinerea* on lettuce (1) and on red clover (10), *Sclerotinia sclerotiorum* on several hosts (10), *Cladosporium fulvum* on tomato (21) and *Mycelium radicis nigrostrigosum*, which usually occurs as a mycorrhizal fungus, on Norway spruce seedlings (7). All these fungi may be facultative parasites, the pathogenicity of which seems, as a rule, to be increased by shading the host.

Different light conditions always result in differences in temperature and moisture. Because 1 or more species of the common soil fungi tested were strongly pathogenic at various temperatures from 7° to 30° C., probably damping-off of tree seedlings occurs at most temperatures suitable for seedling development.

Dryness may sometimes decrease the incidence of

this disease. Roth and Riker (12) believed this to be the case in an experiment where damping-off was more severe under oak and locust canopies than on an exposed site which was usually very dry. Roth (11) has mentioned *Fusarium* epidemics even on open forest land, speculating that the seedlings were predisposed by a certain combination of hot and moist weather.

The effect of shade on damping-off is most important on fertile sites where either the canopy or the ground vegetation is usually dense. Since there the amount of litter is large, seeds on the ground often become completely covered. This will probably cause, as the results in Tables 2-4 suggest, increased seed mortality. Poor sites, on the other hand, seldom have a heavy shade over seeds and seedlings and even then damping-off seems to be negligible.

The mortality of birch was very high on various types of humus when the seedbeds were subjected to short daily illumination and low temperatures (Tables 2-4). This high mortality may have been indirectly caused by inhibition of germination and perhaps could be reduced if one used stratified seed which germinated rapidly. However, even birch seedlings died in great numbers under short daily illumination.

Several species of birch regenerate easily after fire but with difficulty under most other conditions. On fertile sites even Scotch pine has the same difficulty. The role of burning in decreasing damping-off has been demonstrated with Norway pine in pot cultures (8). Fire changes seedbeds in many ways, 2 of which are likely to effect damping-off directly (19): (1) killing parasites, (2) destroying residues which serve as substratum for facultative parasites, and (3) indirectly by reducing shade over seedbeds.

Damping-off has caused failures in direct seedings of ponderosa pine in British Columbia and may limit the distribution of the species (17). According to Wilde and White (23) this disease confines red pine to poor sites in Wisconsin. These hypotheses are supported by the results of the present study. It is possible that damping-off influences the regeneration of many other species on fertile sites. Probable damping-off is also one of the most important ultimate agencies of mortality among the dense crops of seedlings which may germinate but rarely survive under heavy shade.

Some of the parasites isolated are known also as saprophytes. In these experiments they acted as parasites only under certain conditions, as, for instance, *Botrytis*. Under other conditions this fungus is beneficial because of its antagonistic effect on *Rhizoctonia* and *Pythium*. The absence of these in tested humus perhaps arose from the common presence of *Botrytis*, *Trichoderma* and others. Damping-off by *Rhizoctonia* and *Pythium* is evidently rare in Finnish forest soils, if it occurs at all. In certain Wisconsin forests these fungi cause damping-off (12).

SUMMARY

Pinus silvestris and *Betula verrucosa* suffered no marked damping-off on seedbeds of sand and humus from different forest types in experiments conducted outdoors where the illumination was strong.

Under low illumination intensity in the laboratory, damping-off of many Finnish tree species was dependent on the quality of sites from which the humus was collected. The more fertile the humus used as a seedbed or as an amendment to seedbeds of sand and humus, the greater was the mortality of seed and seedlings. Mortality on humus from fertile *Oxalis-Maianthemum* or *Oxalis-Myrtillus* types was often 100 per cent.

Damping-off was also dependent on the daily duration of illumination if intensity and temperature were low. This was true especially of birch and to a lesser extent of pine. In some cases under short daily illumination, birch seed failed to germinate and died on all humus types.

It was proved that at least 9 and probably more of the fungi isolated from seedlings could be pathogens. Those most commonly isolated were *Papulospora* sp., *Fusarium* spp., *Botrytis* sp. and *Mucor ramannianus*.

In pure culture tests using *Pinus silvestris* as a host and the 3 isolated fungi as pathogens the relation of illumination conditions and damping-off was confirmed.

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PRODUCTION AND PURIFICATION OF THE ANTIBIOTIC HELIXIN[1]

R. R. Smeby, Curt Leben, G. W. Keitt, and F. M. Strong

In an abstract submitted for publication in October 1950, Leben, Stessel, and Keitt (4) reported the isolation of an unidentified *streptomyces* species that produced an antibiotic, which, in partly purified form, was capable of inhibiting all of the 16 fungi and some of the bacteria tested. A later report (5) described in detail the organism and the properties of the partly purified antibiotic. The antibiotic was named helixin, and a unit of activity was defined in terms of an arbitrary standard.

The work of Gottlieb *et al.* (2) describing the partly purified antibiotic endomycin appeared when this second report of Leben *et al.* (5) was ready for publication. It was apparent at that time that helixin and endomycin, and the organisms that produced these antibiotics, were similar, but further researches were necessary to establish the degree of similarity between them. The present paper reports the results of comparative tests with the 2 antibiotics and presents further information on the production, purification, and properties of helixin.

Experimental.—*Fermentation and assay methods.*—Routine determinations of helixin activity were carried out[2] with *Glomerella cingulata* (Stoneman) Spauld. and v. Shrenk (5). Shake flask fermentations were performed in 500 ml. Erlenmeyer flasks containing 100 ml. of medium and plugged with cotton. Foaming was controlled by the addition of 0.1-0.2 ml. of a 10 per cent D. C. Antifoam A[3] suspension in soybean oil. The sterilized (120° C.) flasks were inoculated with 2 ml. of a spore suspension prepared from a 15-day-old test tube culture of the helixin-producing organism grown at 26-28° on yeast extract agar[4]. Suspensions were diluted to give 20–30 per cent transmission on an Evelyn colorimeter with a 660 mμ filter. The inoculated flasks were incubated at 26° on a rotary shaker operated at 105 r.p.m. Activity and pH measurements were made on the culture filtrate every second day. Tests to check for bacterial contamination in the flasks were run every fourth day.

The crude concentrates used in this work were obtained by fermentation in the 100 gal. tank previously described (7). The tank contained, after sterilization at 120°, 220 l. of medium 22; see Table 1. The inoculum was developed by 2 successive shake flask fermentations using medium 1 (Table 1) in the following way. A transfer was made from a stock soil culture to 100 ml. of medium in a 500 ml. Erlenmeyer flask, which was shaken on a reciprocating shaker for 7 days at 26°. This was then divided among four 4-l flasks each containing 1 l. of medium. These cultures were shaken in the same manner for 7 days and pooled for the inoculum. Medium in the tank was maintained at 28-30° during the fermentation, and sterile air was forced through the medium at a rate of 170 l./min. Foaming was controlled by the addition of 600 ml. of the antifoam suspension prior to sterilization. Sterility tests were made daily.

Purification procedures.—Descending paper chromatograms were run on ½ in. Eaton-Dikeman No. 613

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[3] A silicone product of the Dow-Corning Company, Midland, Michigan.
[4] Per l.: yeast extract 3 gm., glucose 10 gm., and agar 17 gm.

TABLE 1.—*Effect of different media on helixin production in shake flasks*[a]

Medium No.	Ingredients	Peak Activity in units/ml.	Days needed to reach peak activity
1	4% SOMI[b], 2% Cerelose[c]	13.5	8
2	4% SOM2[b], 2% Cerelose	16.1	8
3	4% SOM3[b], 2% Cerelose	12.7	10
4	4% SOM4[b], 2% Cerelose	12.5	10
5	4% SOM5[b], 2% Cerelose	13.9	8
6	4% extracted soy flour[d], 2% Cerelose	6.4	4
7	4% lo-fat soy flour[d], 2% Cerelose	6.9	4
8	4% special nutrient[d], 2% Cerelose	8.9	8
9	4% linseed oil meal, 2% Cerelose	8.5	6
10	4% SOMI, 0.5% crude casein, 2% Cerelose	7.0	8
11	4% SOMI, 0.5% extracted casein, 2% Cerelose	11.0	8
12	4% SOMI, 0.5% Bacto peptone, 2% Cerelose	4.2	6
13	4% SOMI, 0.5% yeast extract, 2% Cerelose	2.3	12
14	4% ground soybeans, 2% Cerelose	2.8	4
15	4% corn steep liquor, 2% Cerelose	9.0	10
16	2% SOMI, 0.5% corn steep liquor, 2% Cerelose	3.5	8
17	4% SOMI, 2% brown sugar	4.6	6
18	4% SOMI, 2% lactose	2.9	4
19	4% SOMI, 5% lactose	2.5	8
20	4% SOMI, 2% corn starch	3.0	8
21	4% SOMI, 5% Cerelose	11.0	10
22	4% SOMI, 2% Cerelose, 0.2% sodium nitrate	8.3[e]	8
23	4% SOMI, 2% Cerelose, 0.1% disodium phosphate	12.5[e]	6
24	4% SOMI, 2% Cerelose, 0.2% magnesium sulfate	3.6[e]	10
25	4% SOMI, 2% Cerelose, 0.2% calcium carbonate	8.8[e]	10
26	4% SOMI, 2% Cerelose, 0.2% sodium chloride	10.0[e]	10
27	4% SOMI, 2% Cerelose, 0.2% ammonium acetate	0	14

[a] All values represent averages of from 2 to 4 replicate flasks.

[b] Soybean oil meal samples containing 44 per cent protein supplied by the Archer-Daniels Midland Company, Minneapolis: SOMI, special heat treated meal; SOM2, undercooked meal; SOM3, toasted meal; SOM4, overcooked meal; SOM5, untoasted meal.

[c] Glucose monohydrate produced by Corn Products Refining Company, Argo, Illinois.

[d] Supplied by the A. E. Staley Manufacturing Company, Decatur, Illinois.

[e] Media 22-27 inclusive, were not tested at the same time as the others, but medium 1 was present in this series as a control medium. The maximum activity with medium 1 in this series was 8 units/ml. in 8 days.

paper strips 46 cm. long. The sample to be examined, either 0.4 or 0.2 mg. of solids in 0.02 ml. of ethanol, was placed on the strip, and the solvent was allowed to evaporate. The smaller amount of sample gave the same shape inhibition zones, but they were smaller in size. The strips were equilibrated in a sealed chamber against water for 1 hour, and then in another chamber against 1:1 *n*-butanol-ethyl acetate saturated with water for 1 hour or longer. They were then developed by downward flow of the butanol-ethyl acetate mixture until the solvent front arrived within 3-5 cm. of the bottom of the strip (12-14 hours). All paper chromatograms were run at 23-25°. Small variations in temperature had no apparent effect.

To locate the active zones after development, the strips were cut in half lengthwise, air dried, and ½ of the strip was placed on the regularly seeded assay medium (5) in large flat containers as described by Karnovsky and Johnson (3). When quantitative estimations of the relative amounts of each component were desired, the other half of the strip was cut into ¼ in. squares which were assayed individually (3). Known amounts of the antibiotic in similar dried, ¼ in. squares were used as the standard.

Columns for partition chromatography were prepared from a slurry of 20 gm. of silicic acid[5] in 10 ml. of 0.1 M phosphate buffer, pH 7.0, plus 150-200 ml. of a 15 per cent solution of *n*-butanol in ethyl acetate. The slurry was poured into a chromatograph tube with an inside diameter of 20 mm. When packed under an air pressure of 20 cm. of mercury, columns 130-135 mm. high were produced. Samples (about 30 mg. of solids) were best introduced by dissolving them in 0.5 ml. of the phosphate buffer, mixing with 1 gm. of the silicic acid suspended in 20 ml. of the organic phase, and packing on top of the prepared column. The columns were then developed with the butanol-ethyl acetate mixture used in their preparation. The flow rate was about 1 ml. per min.

RESULTS.—*Production of helixin.*—The helixin-producing organism was found to grow well in shake flasks and fermentation tanks on a soybean oil meal-glucose medium (4, 5) similar to that used for the production of antimycin (1). Since the yield of antimycin was found to vary greatly depending on the ingredients of the medium, especially the type of soybean oil meal used (6), efforts were made to find a

[5] Mallinckrodt analytical grade, 100 mesh, prepared for chromatographic analysis by the method of Ramsey and Patterson.

TABLE 2.—*Procedure used for obtaining preparation II*

Step	Treatment	Fraction tested	Total activity in units	Type of helixin	Units/mg. dry wt.
1.	Eight gm. of preparation I added to 250 ml. absolute ethanol. Filtered.	Filtrate	12,000	A,B,C	1.5
		Residue	0	.....	.....
2.	250 ml. water added to filtrate. Filtered.	Precipitate	0	.....	.....
3.	Filtrate extracted 3 times with ⅓–½ volumes of ethylene dichloride.	Extract	900	C	0.8
4.	Aqueous ethanol layer concentrated to 250 ml. (under reduced pressure) and $NaHCO_3$ added to dissolve precipitate. Extracted with diethyl ether.	Extract	21	.....	.....
5.	Aqueous layer acidified to pH 3. Filtered.	Filtrate	0	.....	.....
6.	Precipitate taken up in 100 ml. absolute ethanol. 200 ml. ethyl acetate added. Filtered.	Precipitate	21	.....	.....
7.	Filtrate taken to dryness (under reduced pressure) resulting in 4.12 gm. of a yellow glass. Preparation II.	.....	11,100	A,B	2.7

medium more suitable for helixin production. The results are summarized in Table 1. The peak activity was generally reached in 6-10 days, and the pH usually rose from 5.3-6.3 to 8.1-9.1.

Purification of helixin.—The 220 l. fermentation described above reached a peak activity of 9 units/ml. in 6 days, and the pH rose from 6.9 to 7.95. An active concentrate was prepared from the culture filtrate by the procedure of Leben *et al.* (5). The crude, orange glass obtained, designated preparation I, contained 1.5 units/mg., and represented 25 per cent recovery of the original activity. However, recovery from 1-2 l. batches of culture broth from shake flask fermentations ranged from 41-63 per cent. Preparation I has been stored for 8 months at room temperature without loss of activity. It was further purified as shown in Table 2 to give a yellow glass, preparation II, which had an activity of 2.7 units/mg.

A somewhat shorter procedure, as yet not fully evaluated, has yielded products comparable in purity to preparation II. The gum obtained by Leben *et al.* was washed with ether, taken up in 0.1 per cent aqueous ammonia, and extracted with *n*-butanol. The aqueous phase was discarded, and the butanol was removed under reduced pressure. This yielded an active yellow glass that contained helixins A, B, C, and D.

In 52 tests preparation I was found by paper chromatography to contain 3 active components having average R_f values of 0.07, 0.42, and 0.85 (Fig. 1). These were named helixins A, B, and C respectively[6]. On about every fifth paper chromatogram of preparation I, a trace of another active substance with an R_f value of approximately 0.68 was noticed. Preparation II was found by paper chromatography to contain helixins A and B (Fig. 1). Helixin C was removed from preparation I by the ethylene dichloride extraction (Table 2), and it was recovered as an oil (0.8 units/mg.) free from helixins A and B on the removal of the solvent.

With both preparations I and II only 50 per cent of the total activity put on the paper strips could be accounted for when the quantitative estimations for each component were carried out. Of the 50 per cent recovered, preparation I was found to contain 22.6 per cent of component A, 72.7 per cent of component B, and 4.7 per cent of component C. Preparation II contained 21.2 per cent of component A and 78.7 per cent of component B. These results are shown in Fig. 1.

When 1 gm. of preparation II was dissolved in 100 ml. of 0.06 aqueous ammonia and extracted with 1:1 *n*-butanol-ethyl acetate, about 0.5 gm. of a yellow glass was obtained from the organic layer. This fraction, preparation III, had an activity of 1.9 units/mg., and was found by paper chromatography to contain the B component, but none of component A. However, it was accompanied by a small amount (*ca.* 1-2 per cent of the total activity) of a fourth active substance, helixin D, R_f 0.71. Presumably this D component was the active substance with R_f 0.68 occasionally noted in chromatograms of preparation I.

The aqueous layer remaining after the butanol-ethyl acetate extraction described above was extracted with *n*-butanol, and from the organic layer a second yellow glass (preparation IV) was obtained. This product contained only helixin A (2.2 units/mg.) as judged by paper chromatograph.

The helixin B in preparation III was further purified by means of partition chromatography on a silicic acid column using a 15 per cent solution of *n*-butanol in ethyl acetate as the outside phase. The effluent from the column was collected in 20 ml. fractions, each of which was analyzed for total solids and antibiotic activity. Fifty per cent of the activity placed on the column was found in fractions 20-23. From these fractions a colorless powder containing 5.9 units/mg. was obtained. Material of a lesser degree of purity,

[6] The R_f values are only approximate since the zones (especially that of helixin B) were considerably elongated (Fig. 1). The R_f values were calculated from the middle of each zone.

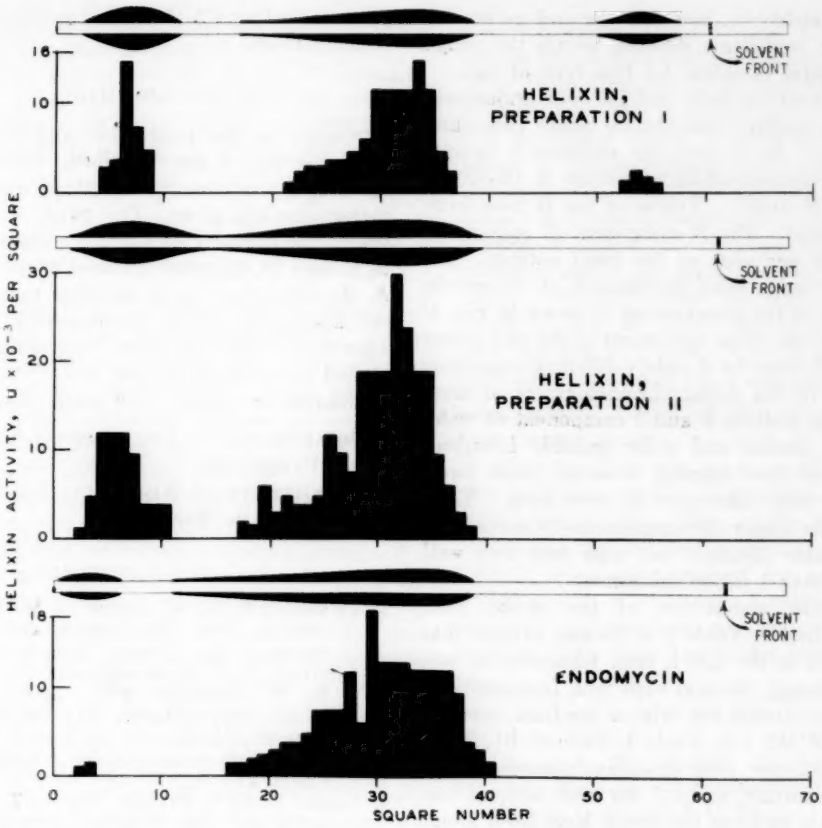

FIG. 1. Examples of paper chromatograms of helixin preparations I and II and of endomycin. The samples were applied at the point "O." The bar graphs represent the relative activity in the ¼ in. squares cut from the paper strips.

representing 32 per cent of the total activity, was obtained from fractions 24-30.

Comparison of helixin and endomycin.—Two series of agar-streak tests were made with 8 microorganisms to compare the inhibitory effects of helixin and endomycin[7]. The helixin sample used was one prepared in earlier work (5), and was found by paper chromatography to contain components A and B. The relative proportions, as judged by the gross appearance of the chromatogram, were about the same as in preparation II. In these tests varying amounts of the antibiotics were incorporated in warm potato agar and poured into plates. The test organisms were streaked on the surface of the hardened agar. The methods and materials used in making the tests have been described elsewhere (5). The pH of all test and control media was between 5.8 and 6.0. Data were taken after 4 days at 24° (Table 3). Results show that the end points for 6 of the organisms were the

[7] We are indebted to Dr. David Gottlieb of the University of Illinois and Dr. Jared H. Ford of the Upjohn Company for the sample of endomycin used. This sample (Res. No. 87-GCP-6) was stated to contain 1350 unit/mg. as assayed by the cup-plate assay using *Candida albicans* (Robin) Berkhout and the University of Illinois preparation III-30-III as the standard (personal communication from Dr. Ford). Assay of this sample by the *G. cingulata* method used in the present work showed 2.1 units/mg. of activity based on the helixin standard.

TABLE 3.—*The inhibition of 8 microorganisms by helixin and endomycin. Agar-streak tests*[a]

Microorganism	End point[b] in μg./ml. for Helixin	Endomycin
Alternaria solani (E. and M.) Jones and Grout	2.8	2.8
Bacillus subtilis Cohn *emend.* Prazmowski	5.6	11.2
Candida albicans (Robin) Berkhout	22.5	22.5
Corynebacterium fascians (Tilford) Dowson[c]	11.2	11.2
Fusarium oxysporum f. *lycopersici* (Sacc.) S. and H.	11.2	11.2
Glomerella cingulata (Stoneman) Spauld. and v. Schrenk	2.8	2.8
Verticillium albo-atrum Reinke and Berth.	2.8	2.8
Xanthomonas campestris (Pammel) Dowson	5.6; 11.2	5.6

[a] Each entry on the basis of 2 series of tests; where 2 figures appear the series differed.

[b] Smallest amount (dry weight) that completely prevented macroscopic growth.

[c] Not previously reported to be inhibited by helixin.

same for both antibiotics, and that the end points for the 2 remaining organisms differed within the range of the experimental variation for this type of test.

Since by agar-streak tests helixin and endomycin appeared to be similar, comparative paper chromatograms were run. In 2 tests the endomycin sample produced zones corresponding to helixin A (R_f 0.07) and helixin B (R_f 0.41). Traces of the D zone were sometimes observed. The B component of endomycin represented 98.6 per cent of the total activity. An example of the comparative distribution of endomycin and the helixins on the paper strips is shown in Fig. 1.

On the basis of the close agreement of the end points in the agar-streak tests for 8 widely differing organisms and the results of the paper chromatograms, it must be concluded that helixin B and 1 component of endomycin are very similar and quite possibly identical. Absolute proof of their identity however, must await the isolation of both substances in pure form. Since the B zone on the paper chromatograms is spread out over a considerable distance, this zone may very well represent more than 1 active substance.

DISCUSSION.—On completion of the shake flask studies, summarized in Table 1, it became evident that the medium used in the 220 l. tank fermentation was probably not optimal. Several such tank fermentations have since been carried out with a medium containing 4 per cent SOM2 (see Table 1, footnote b), 2 per cent Cerelose, 0.2 per cent disodium phosphate, 0.2 per cent sodium nitrate, and 0.2 per cent calcium carbonate. With this medium the yields have been about double those obtained earlier, and the fermentations were completed in 4 to 6 days.

In the present work the similarity between 1 of the components of endomycin and helixin B has been noted. In the endomycin fermentation Gottlieb *et al.* (2) have noted the presence of 2 other antibiotics. No conclusion can be reached at this time as to whether 1 or both of these substances are related to the minor products helixins A, C, and D produced in the helixin fermentations.

SUMMARY

Studies on the production and purification of the antibiotic helixin are described. Systems for chromatographing the material on paper and partition chromatograms are given. The paper chromatograms revealed the presence of 4 active components, helixins A, B, C, and D, in partly purified preparations. Helixins A, B, and C have been obtained essentially free from one another by solvent fractionation. On the basis of agar-streak tests and paper chromatograms, it was concluded that helixin B and one of the components of endomycin are similar and quite possibly identical.

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A PHYTOPATHOLOGICAL NOTE

Crown Bud Rot of Alfalfa.[1] E. J. HAWN AND M. W. CORMACK. Crown bud rot is the name now proposed for a hitherto undescribed disease complex of alfalfa which is of considerable economic importance in the irrigated areas of southern Alberta. This disease, in contrast to the previously described winter crown rot of dormant plants,[2] occurs during the growing season. It causes progressive deterioration of alfalfa stands after the first season of growth. Brown lesions first appear on the buds and young shoots below and at ground level. These lesions develop into a rot which kills infected buds or shoots and may eventually extend into the underlying crown tissues. As infection progresses in plants throughout the field, the crowns become partially devoid of buds and stems, with consequent loss in yield.

Information on the distribution and importance of the disease was obtained by taking replicated samples at regular intervals in representative fields in the 5 main alfalfa growing areas of southern Alberta. The percentage of infected plants was determined at each sampling and the individual plants were rated for crown bud damage on a percentage basis. The disease was prevalent in all irrigated alfalfa fields 1 year old or older examined, and occurred much less commonly in non-irrigated fields. Data obtained during 2 years

[1] Contribution No. 1178 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Cormack, M. W. 1948. Winter crown rot or snow mold of alfalfa, clovers, and grasses in Alberta. 1. Occurrence, parasitism, and spread of the pathogen. Can. Jour. Res. C 26: 71-85.

TABLE 1.—*Progress of crown bud rot infection in irrigated alfalfa stands of different ages*

Year of planting	No. of fields studied	Percentage of plants infected		Percentage of damaged buds	
		1950	1951	1950	1951
1947	4	100	100	45	57
1948	10	99	100	46	52
1949	10	76	94	12	42
1950	2	1	51	0	19

on irrigated fields (Table 1) show that an average of 50 to 75 per cent of the plants were infected during the second season of growth, and nearly 100 per cent by the third season. Similarly, the disease rating of the individual plants increased with age, and approximately 50 per cent of the crown buds were destroyed on the plants in stands 3 to 4 years old.

Extensive isolation studies on diseased material indicate that a complex of soil inhabiting fungi are involved in crown bud rot damage. During 2 years, the fungi isolated were as follows: *Fusarium acuminatum* Ell. and Ev.—42 per cent, *F. avenaceum* (Fr.) Sacc.—9 per cent, *F. culmorum* (W. G. Sm.) Sacc.—2 per cent, *F. poae* (Peck) Wr.—2 per cent, *Rhizoctonia solani* Kühn—28 per cent, *Ascochyta imperfecta* Peck—10 per cent, miscellaneous fungi (mainly *Plenodomus meliloti* Dearness and Sanford, *Colletotrichum* sp., and *Alternaria* sp.)—7 per cent.

F. acuminatum, *F. avenaceum*, and *Rhizoctonia solani* were consistently associated with the disease in all irrigated fields examined. In greenhouse and field infection studies these 3 fungi proved pathogenic on alfalfa crown buds and each produced symptoms similar to those occurring in nature. They were readily reisolated from the diseased tissues. Maximum infection developed within 2 weeks on 3-month-old alfalfa plants in the greenhouse when inoculum grown in a soil-corn meal medium was scattered among the crowns and lightly covered with soil. Under these conditions, *R. solani* proved more virulent than *F. acuminatum* and *F. avenaceum*. The damage was significantly increased when inoculum of *R. solani* was added to that of either species of *Fusarium*. Infection has not yet been obtained in inoculation tests with any of the other fungi isolated.

In previous studies,[3] *F. avenaceum* and *F. acuminatum* proved parasitic on roots of alfalfa in Alberta. *R. solani* has been frequently reported as causing parasitic symptoms on alfalfa, including crown rot in Manitoba,[4] and a crown bud blight in North Carolina.[5] However, a definite disease of the crown buds in which several fungi are involved has apparently not received previous attention. Further studies, including host and varietal tests, are in progress.—Laboratory of Plant Pathology, Lethbridge, Alberta.

[3] Cormack, M. W. 1937. Fusarium spp. as root parasites of alfalfa and sweet clover in Alberta. Can. Jour. Res. C 15: 493-510.

[4] Cherewick, W. J. 1948. Two important diseases of alfalfa in Manitoba. Phytopathology (Abs.) 38: 5.

[5] Hanson, C. H. and J. L. Allison. 1951. Studies on the nature and occurrence of stand depletion in alfalfa strains in North Carolina. Agronomy Jour. 43: 375-379.

REPORT AND ABSTRACTS OF THE THIRTY-FOURTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The thirty-fourth annual meeting of the Pacific Division of the American Phytopathological Society was held at Oregon State College, Corvallis, Oregon, June 19 to 21, 1952, in conjunction with the annual meeting of the Pacific Division of the A.A.A.S. Included on the program were 70 papers, symposia on "Nematodes" and "Legume Viruses," and a demonstration session. The Phytopathologists' Dinner on Friday evening, June 20, was attended by 115 members and guests. A field trip to the Plant Pathology Department Farm and Field Laboratory and to points of interest in the Willamette Valley concluded the meeting on June 21. Registration of 118 plant pathologists included representation from Alberta and British Columbia, Canada; from California, Colorado, Idaho, Hawaii, Oregon, Maryland, Utah, and Washington.

Officers elected for the year 1952-53 were: *President*, E. K. VAUGHAN; *Vice-President*, GILBERT L. STOUT; *Secretary-Treasurer*, ROY A. YOUNG (2-year term); and *Councilor*, WILLIAM B. HEWITT (2-year term).

GEORGE A. ZENTMYER,
Secretary-Treasurer.

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Preliminary trials on chemical roguing of virus-diseased potato plants. BARNES, GEORGE L. Data from aphid control trials in Oregon during recent years have indicated that hand roguing of virus-diseased potato plants has not prevented spread of these diseases. Scattering of aphids to healthy plants may result during roguing. Chemical roguing was proposed to prevent this occurrence and to reduce roguing costs. During 1950, a variety of herbicides were screened in greenhouse and field trials. Three very effective materials were found: they are Shell 30; a mixture of 1 gal. of Dow General Weed Killer (55 per cent dinitro-o-secondary-butyl phenol) and 100 gal. of diesel oil; and diesel oil. Field trials were run in 1951 to determine which material would be most satisfactory in regard to rapidity of kill, cost, and amount of regrowth. The mixture of Dow General Weed Killer and diesel oil proved to be the most

effective and is both insecticidal and herbicidal. Only about 25 per cent regrowth occurs after treatment with this material. A repeat spraying controls this regrowth. Further work is being done on developing a mixture which will kill the tops, seed pieces, and new tubers.

Fungicidal effect of the sodium salts of certain chlorinated phenols on the sclerotia of Rhizoctonia solani on potato tubers. BARNES, G. L. AND J. H. MUNCIE. The use of formalin and certain proprietary mercury compounds for treating whole potato tubers to control common scab and *Rhizoctonia* scurf has many disadvantages. These treatments frequently cause damage to sprout primordia and cause a reduction in stand or a delay in emergence. The mercurials are toxic to animals, corrosive to metals, and are costly. Mercury-tolerant Streptomycetes also develop in the soil after their continued use. The low cost Dowicides (sodium salts of chlorinated phenols, phenyl phenols, and chlorinated phenyl phenols) were screened to determine their efficiency in killing sclerotia of *R. solani*. Whole tubers, bearing sclerotia of various sizes, were treated. The sclerotia were aseptically removed and placed on potato dextose agar to determine their viability after treatment. Dowicides B, C, F, and G were found to be more effective than the standard tuber fungicides. Dowicide F (sodium tetrachlorophenol) is the most suitable; it is very efficient at high dilutions, and more suitable in many respects than the standard tuber fungicides.

Index tests for virus in Myrobalan Plum. BLODGETT, E. C., H. E. WILLIAMS, T. O. DIENER, AND J. G. BARRAT. Three budsticks from each of 161 Myrobalan Plum (*Prunus cerasifera*) seed trees, representing 6 sources in Washington and 1 source in California, were collected, and 1 bud from each stick was indexed on Shiro-fugen flowering cherry. One hundred fifteen trees or 71.4 per cent gave a negative reaction, 27 trees or 16.8 per cent gave a mild reaction, 2 trees or 1.2 per cent gave a moderate reaction, and 17 trees or 10.6 per cent gave a severe reaction on Shiro-fugen. Twenty-seven trees or 52.9 per cent of the California source reacted with Shiro-fugen, whereas only 19 trees or 17.3 per cent of the Washington sources gave a positive reaction. Attempts were made to transmit virus mechanically from 21 reacting Myrobalan trees to National Pickling cucumber plants, using immature leaves ground in M/100 phosphate buffer (pH 7.4) as inoculum. As a control, 5 nonreacting Myrobalan trees were included in the test. No symptoms were observed on any of the inoculated cucumber plants.

Control of brown rot of lemon fruits by various fungicides. CALAVAN, E. C., L. J. KLOTZ, T. A. DEWOLFE, AND J. R. SUFFICOOL. Twenty-five spray formulations applied to lemon trees near Oxnard, California, in November, 1951, were evaluated for the control of *Phytophthora citrophthora* by lesion counts of inoculated fruits. After 10 weeks and 15.85 in. of rain the 6 materials (with pounds per 100 gal. in parentheses) giving best control, in descending order, were: Orthocide 406 (4), Orthocide 406 + Z-1 spreader (2—0.25), Crag 640 (2), zinc-copper lime (2-1-2), Bordeaux mixture (3-3), Copper A Compound + lime + DuPont spreader-sticker (1.5-3-0.125). Orthocide 406, which was not injurious at the 2-lb. and only slightly so at the 4-lb. level, provided the best field control thus far obtained from a non-copper material. Ferric-zinc Dithane D-14 (1 lb.-1 lb.-1 gal.), the only other non-copper material providing commercial control, ranked thirteenth and caused some injury to ripe fruits. The standard treatment, zinc-copper-lime (5-1-4), gave excellent control for 6 weeks but ranked seventeenth after 10 weeks. Copper-containing materials injured the foliage in varying degree.

Renewal of shell-barked lemon trees by pruning. CALAVAN, E. C. AND J. R. SUFFICOOL. Lemon trees which have deteriorated from severe shell bark (dry bark) may usually be renewed by heavy pruning. Near Ventura, California, 93 per cent of 1123 badly diseased Eureka lemon trees pruned February to July, 1948, responded satisfactorily within 10 months. In August, 1951, 76 per cent remained in fair or good condition. Additional pruning was done as needed. Yield estimates from 388 pruned trees for the 1949-50 season indicated a normal crop on 36 per cent. Thirteen per cent had less than ⅓ normal production. Average production of pruned diseased trees was estimated as 70 per cent that of unpruned normal trees. The most satisfactory pruning height for diseased trees was found to be 5.5 to 8 ft. The most favorable response was obtained from trees pruned during the period of March to July, inclusive. Fall or winter pruning predisposed diseased trees to severe frost damage.

Bud rot of rhubarb. CAMPBELL, LEO. In rhubarb, as in other species of the Polygonaceae, the stipules cover each bud as a protective sheath. During the winter, however, the sheaths covering the oldest buds gradually decay, exposing the young leaves to the weather and invading fungi. As a result, the upper portion of these leaves are decayed, leaving at the most only a few inches of the lower portion of the petiole as a necrotically-tipped stub. These leaves are the earliest and choicest product, hence yields would be considerably increased if this decay could be prevented.

Interference between forms of the ring spot virus in peach trees. COCHRAN, L. C. Earlier (Phytopathology 36: 396. 1946.) it was reported that forms of the peach mosaic virus producing mild effects variously protected peach trees against effects of forms which alone produce severe symptoms. Preliminary results indicate similar reaction between forms of the ring spot virus. The severity of symptoms caused by the ring spot virus apparently depends on certain environmental factors such as temperature, vigor of host plant, time of year of infection, and others. Seven forms, selected for mild symptom production but producing varying severity, were matched with 3 forms known to produce severe symptoms. Inoculations with the "mild forms" were made in September 1950 and reinoculations with "severe forms" were made in September 1951. No symptoms resulted from reinoculations on trees in which the original inoculations caused both leaf symptoms and bark necrosis. Three of the "milds" afforded protection against 1 of the "severes" but were severely shocked by the other 2. Symptoms were less severe than on trees which had not been infected with "mild." It thus appears that ring spot virus forms afford varying protection against each other. Forms which cause stem necrosis following inoculation into rapidly growing peach nursery trees in August are most likely to give the strongest protection.

Virus invasion of tops of certain naturally immune stone fruits when grown on infected rootstocks. COCHRAN, L. C. Graft inoculation of suspect species, followed by recovery onto a known susceptible test plant, has been the standard technique for determining the host range of stone fruit viruses. Indexing of sweet cherry orchard trees growing adjacent to peach mosaic-affected peach has never yielded the virus. The virus was recovered from a cherry shoot grown on Hungarian prune, which was in turn growing on a mosaic-infected peach tree. Growth from buds from this same cherry shoot, which had been placed on a mazzard cherry seedling, was free of virus 1 year later. Peach mosaic virus was likewise recovered from *Prunus fremonti* and *P. fasciculata*, growing on infected peach, but was not recovered from inoculated seedlings of these species. Virus recovery is consistent from horticultural plums which produce symptoms, but is erratic from others which develop no symptoms. Such symptomless varieties may be immune but virus sometimes moves into the plum tops from susceptible rootstocks. When ring spot virus was introduced into immune Burbank plum growing on myrobalan rootstock, the virus survived sufficiently to move down into the susceptible rootstock. It appears necessary to grow test plants on their own roots or on immune rootstock for determining susceptibility or immunity.

Variation in mode of host penetration by lettuce downy mildew. COHEN, MORRIS. Where several reports of host

penetration mode by a species of the Peronosporales have been made, the several authors have usually differed in their observations. The present quantitative study of host penetration by certain of the Peronosporales helps to explain these apparent contradictions and may be illustrated by the portion devoted to lettuce downy mildew—*Bremia lactucae*. Some authors report stomatal penetration by the germ tubes of this fungus; others report direct penetration. Detached leaves of greenhouse-grown Bibb and Romaine varieties of lettuce were atomized with a water suspension of sporangia, incubated in Petri dish moist chambers at definite temperatures, cleared in chloral hydrate solution, and observed microscopically for numbers of stomatal and direct penetration. With few exceptions the percentage of stomatal penetration was greater through the upper leaf epidermis than through the lower epidermis. When moist chamber incubation temperature, leaf age, and morphology were allowed to vary simultaneously, trends in percentage of stomatal penetration were discernable, the values ranging from 0 to 100 per cent. With Romaine lettuce the percentage of stomatal penetration was greater than with Bibb lettuce, but interpretation of this result is complicated by the present inadequate understanding of physiological leaf age as regards host penetration.

Inactivation of phenyl mercury triethanol ammonium lactate by colloidal clays in vitro. CULVER, ANDREW J., JR. Puratized Agricultural Spray was diluted to 1 ppm. To 100 cc. of this dilution was added 0.5 gm. of either Panton clay loam or Vergennes clay loam, and the mixture stirred for ½ hour. The LD 50 values and slope of the mixtures were determined by the standard A.P.S. slide germination test method (test-tube dilution technique). The average of 10 replicated tests show the untreated (no clay) 1 ppm solution had an LD 50 of 0.312 ppm, Panton clay loam 1.4 ppm, and Vergennes clay loam 1.0 ppm. The corresponding slopes were .90, .58, and .64. From the standpoint of the LD 50 data it appears that the Panton clay loam lowered the effectiveness of *Puratized* by 4 times, the Vergennes clay loam by 3 times.

Unusual aspects of Claviceps investigations. CUMMINGS, R. H., C. M. WRIGHT, AND SHERL M. DIETZ. Alkaloids derived from ergot sclerotia are valuable in the field of medicine. Attempts have been made to grow *Claviceps purpurea* in artificial culture to produce sclerotia with an alkaloid content comparable to that obtained commercially from sclerotia produced on rye plants. Attempted sterilization of natural media with carbon disulfide, ethyl chloride, propylene oxide, and combinations thereof has failed. Other aspects of the investigation have been inoculation of rye stems, and germination of ergot sclerotia of *Eleocharis palustris*. Inoculations of rye stems with honeydew from ergot infected plants were 40 per cent successful. Probably successful inoculation can be made of stems only before flowering. The presence of the head is not necessary for successful stem infection. Successful infection was accomplished at each of the first 3 nodes. The growing fungus is not dependent on the developing seed embryo for nutrients, and sclerotial development can occur away from the point of inoculation. The production of the perfect stage of *Claviceps nigricans* is the first reported in this country. Measurements show much longer ascospores in proportion to ascus size than in *Claviceps purpurea*.

Use of chloropicrin in steam for disinfesting storage houses. DOBIE, NORMAN D., AND ROY A. YOUNG. Chloropicrin has been recommended at the rate of 1 lb. per 1000 cu. ft. for sweet potato storage house disinfestation. This recommendation was based on trials in which chloropicrin was applied to burlap bags distributed throughout a storage house. In an effort to increase the efficiency of chloropicrin and reduce discomfort in handling, trials were conducted in which chloropicrin was introduced into storage houses in steam generated with a flash boiler. The boiler was operated at 500° F. and at 60 lb. pressure. Efficient kill of a test organism was consistently obtained with a dosage rate of ¼ lb. and in most cases at a rate as low as ⅛ lb. per 1000 cu. ft. The time required for application, including raising the temperature and humidity to optimal levels, was from 10 to 15 minutes for an average storage house of 15,000 cu. ft. During the entire operation the operator remained outside the building which made application much less hazardous. The increased efficiency of chloropicrin when applied in steam may be attributed to more rapid dispersion and volatilization due to decrease in particle size and to establishment of optimum conditions for fungicidal action by raising temperature and humidity.

Strains of Botrytis pathogenic to blueberries. EGLITIS, MAKSIS, FOLKE JOHNSON, AND D. C. CROWLEY. A twig, tipblight of cultivated blueberries has become of economic importance in western Washington. The symptoms are characterized by the appearance of light brown, discolored tissue which may extend downward from the twig apex for a distance of less than 1 to approximately 12 in.; old infections later become gray. *Botrytis* sp. has been shown to be the cause. Inoculations were made into wounds in 1-year-old twigs of the Jersey variety, which subsequently were held in a damp chamber. Several strains of the fungus have been isolated from blueberries, as evidenced by different, cultural behavior on P. D. A. media. Botrytis isolates obtained from *Rhododendron* sp., laurel (*Prunus laurocerasus*), raspberry (*Rubus idaeus*), strawberry (*Fragaria* sp.), and *Jasminum* sp. were all pathogenic. Plants grown on peat soils, or those on mineral soils of high fertility appear to be more susceptible to infection than those grown on a lower nitrogen level. On peat soils with a high water table the dead terminal leaves of the succulent shoots remain attached over winter. Botrytis can be recovered from these dead leaves and is believed to be a source of further infections. There is evidence that varieties differ in their susceptibility to Botrytis.

Phomopsis canker of figs. ENGLISH, HARLEY. This disease, caused by *Phomopsis cinerescens* (Sacc.) Trav., has been recognized in California for some time but in recent years it has become especially destructive. Of widespread occurrence in the interior valleys, at present it appears to be causing greatest damage in Merced County. The disease has been found on all important commercial varieties, but is most serious on the Kadota variety, due largely to the fact that Kadota trees are pruned extremely heavily and the pathogen is largely a wound parasite. Three strains of the causal fungus that differ in cultural characteristics and in pathogenicity have been isolated. The optimum temperature for growth in culture was approximately 25° C., with very limited growth at 4° C. and 30° C. Inoculation tests have shown that the fungus can produce rapidly enlarging cankers throughout the dormant season, but that it can cause slight if any infection during the period of active tree growth. Evidence to date suggests that pruning Kadota trees as they are emerging from dormancy, rather than during the winter, may provide an effective means of reducing the severity of this disease.

Pathogenicity of fungi associated with crown rot of alfalfa. ERWIN, DONALD C. *Stagonospora meliloti* (Lasch) Petr. causes a dry rot of alfalfa crowns and roots with a reddish flecking of the infected bark and xylem tissues. The fungus grows slowly in culture. The optimum temperature is between 18° and 21° C. The first symptoms on month-old plants usually appear about 2 months after inoculation. Inoculating the soil with an oat culture of the fungus before planting alfalfa has caused infection of plants not artificially wounded. In 7 tests, an average of 29 per cent of 491 Africa alfalfa plants showed symtoms as compared to 16 per cent of 524 plants of California Common. *Fusarium roseum, F. solani*, and some pycnidial fungi including 1 Phoma sp. have been isolated in 37 experiments from a crown rot characterized by a dark, dry necrosis of the crown branches. None of these isolates caused a root or crown rot when inoculated on plants growing in sterilized soil. *Rhizoctonia solani* and *Pythium spp.*, isolated fre-

quently during the winter from necrotic crown buds, were pathogenic to buds and seedlings. A *Fusarium roseum* isolate, formerly designated as *F. avenaceum*, was severely pathogenic to seedlings but not to crown buds or roots.

Growth variation of bacteria-free crown-gall tumor tissue. FREDERICK, LAFAYETTE, AND SETH B. LOCKE. Experiments have been in progress to determine *in vitro* growth patterns f bacteria-free crown-gall tissue. Considerable growth variation has been found in tumor tissue fragments originating from different tumors, from the same tumor, and from the same culture piece or explant after several passages in culture. These growth variations ranged from very slow (a fresh weight increase of 1- to 2-fold) to very rapid (fresh weight increases of over 10-fold) over 14-day experimental periods. The maximum growth variations are found within explants that have been carried through several passages. Tissue clones that maintain reasonably uniform rates of growth may be separated from these explants. Rapidity of growth appears to be directly related to the maximum wounded area of an explant. Tissue fragments from slow-growing explants frequently become rapid-growing when subcultured as broad, thin slices. The influence that known growth-promoting substances have on natural growth patterns of tumor tissue has been studied. The growth hormones used produced a noticeable, frequently sustained acceleratory effect on tissue growth among rapid-growing clones of tissue. The response of slow-growing clones to growth substances was slight.

Two recently isolated curly top virus strains. GIDDINGS, N. J. An unusually virulent curly top virus appeared in tests with field collections of the beet leafhopper (*Circulifer tenellus*), made during the summer of 1947. The sugar beet variety SL 68, considered extremely resistant, was severely injured by a more virulent strain of curly top virus than any previously isolated. Many of the young plants, when infected with the new strain, died. Some of the more recently developed commercial sugar beet varieties show more resistance to this virus than SL 68. The new curly top virus strain is designated as No. 11. Tomatoes and potatoes from Prosser, Washington, suspected of being infected with curly top, were tested by the usual beet leafhopper technique. A curly top virus strain was obtained that infects potato more readily than any of the strains previously tested. In potato it induces the symptoms that have been known as Green Dwarf. Infected sugar beets are relatively slow in developing symptoms and in the early stages the symptoms are rather difficult to distinguish. There is practically no vein clearing in sugar beet, and leaf symptoms consist mostly in dwarfing and leaf rolling. This virus strain is designated at N-. 12.

Black rot of iris, tulip, and hyacinth bulbs. GOULD, CHARLES J. AND D. M. MCLEAN. An unusual rot of certain ornamental bulbs has been under observation in western Washington since 1946. It appears in bulbous iris early in the spring in the form of a yellowing and wilting of one or more plants. Initial infections appear to take place on the underground shoot near the top of the bulb. The causal organism usually penetrates downward through the outer scales to the base and from there invades the central scales. Underground shoots and bulbs are often covered with a thin, grey mycelium that sometimes mats several bulbs together. At first, the infected iris bulb tissue is very soft, having a water-soaked or dead-white appearance. Later the affected parts become an intermingled mass of bulb tissue, pickets of white mycelium and black sclerotium-like layers. Finally, nothing remains but a residue of decayed bulb fragments and sclerotia. Sclerotia are black, irregularly shaped, often flattened and vary in size from 1 to 10 or more mm. in size. The effect on hyacinths and tulips is, in general, similar to that on iris. Infected daffodil bulbs have recently been collected. Croft lilies were not affected in greenhouse tests. The organism has not yet been identified.

A serious rootlet rot of alsike clover. HARDISON, JOHN R. Premature decline and death of alsike clover, *Trifolium hybridum* L., plants has become a serious problem during the last few years in seed-producing areas under irrigation in central Oregon. Plants may be severely damaged or killed 20 to 24 months after planting. Frequently, many plants in 2-year-old fields die during late spring when growing conditions are favorable. The alsike clover acreage has been greatly reduced because of the risk of unprofitable stands. The trouble is associated with root diseases. One predominate symptom is a rot of rootlets. The rot apparently stops at the base of the rootlets and seldom extends into the lateral or primary roots. When severely infected all the rootlets may be killed. Death of many plants appears to result from the loss of the absorbing root system and to show no other apparent injuries. Although several species of Fusarium and other fungi are encountered, an unidentified species of Phoma has been consistently the most prevalent isolate recovered from rootlet tissue. In artificial soil inoculations the Phoma is the only organism that has reproduced the rootlet disease, and unwounded primary and lateral roots were rarely infected.

Field applications of fungicide reduce decay in stored grapes. HARVEY, JOHN M. There is evidence that the fumigation of grapes with sulfur dioxide kills conidia of *Botrytis cinera* Pers. on the surface of berries and thus reduces the number of infections occurring during storage. Much of the decay which occurs in storage appears to be due to pre-harvest infections which are not controlled by fumigation. An attempt was made to control these pre-harvest infections by applying a protective fungicide, Orthocide 406 (N-trichloromethyl-thiotetrahydrophthalimide), in the vineyard. Field treated grapes and untreated checks were harvested and placed in storage for 16 weeks. A part of the harvest was fumigated with sulfur dioxide at weekly intervals and another portion of the harvest was not fumigated. Examination of the grapes after the storage period showed that decay in unfumigated grapes was reduced by over one half by the pre-harvest application of Orthocide 406. Storage fumigated, field-dusted grapes had only 1/13 as much decay as fumigated, non-dusted grapes. Under the conditions of this preliminary test, therefore, storage decay was reduced significantly by using this fungicide against infections of grapes in the field in conjunction with sulfur dioxide fumigation for control of post-harvest infections.

Transmission of the factor causing "white Emperor" through different grape stocks. HEWITT, WM. B. Fruit on vines with white Emperor fail to develop the red color characteristic of the Emperor variety of grapes; leaves appear thick and roll down at the margins. White Emperor was transmitted to red Emperor grapevines preventing normal color formation of the fruit in 27 out of 31 graft inoculations when wood from white Emperor vines was grafted into young red Emperor vines. Four different varieties of rootstocks, each propagated from a single vine, were grown for a season in vineyard rows and were budded in September of the first season as follows: red Emperor buds from healthy vines were grafted into 8 of the 17 vines of each variety; red Emperor buds were grafted into the remaining 9 stocks of each variety at the ground level; and white Emperor buds were also grafted into each of these rootstocks on the opposite side and about 5 in. lower. The following spring both buds, "red" and "white," were forced and shoots trained upon a trellis. In the third season after grafting, fruit and leaf symptoms showed that the cause of white Emperor was transmitted through the wood of the rootstocks known as 1202, 1613, St. George, and Salt Creek.

Root and rhizome rot of peppermint. HORNER, C. E. During the winter and early spring of 1950-51 and 1951-52 peppermint in Western Oregon was affected by a root and rhizome rot caused by a complex of several fungi. *Rhizoctonia solani, Fusarium roseum, Fusarium* sp. and *Pythium* sp. were consistently isolated and their pathogenicity established. Symptoms appear first on rhizomes and consist of advancing necrotic lesions usually girdling the rhizomes. As the new shoots are formed in the early spring the necro-

sis often advances up the new shoots from the parent rhizome. New shoots are also attacked 2 to 6 in. below the soil resulting in a type of damping off. Fall-plowed mint consistently resulted in stronger stands than spring-plowed mint where root rot was severe. Fall plowing resulted in sufficient new shoots becoming established before the parent rhizomes rotted away. Infection experiments showed that *Rhizoctonia solani* caused progressive necrosis of artificially infected rhizomes at temperatures from 5 to 35° C. while the *Fusaria* and *Pythium* were active from 15 to 35° C.

Studies on flax Fusarium wilt (Fusarium oxysporum f. *lini) in California.* HOUSTON, BYRON R. AND P. F. KNOWLES. A method of inoculation with flax Fusarium wilt was devised for use in the greenhouse to facilitate a rapid development of resistant varieties adapted in California and for use in studies of the organism and disease development. Flax seeds were placed in furrows ¾ in. deep in flats of sterile soil and a water suspension of spores placed on the seed and bottom of the furrow. About 1 cc. of suspension was used for each inch of row. The suspension was prepared by adding distilled water to a 22 mm. test-tube slant culture 10 days of age and scraping the surface to remove the spores. The suspension was strained through cheesecloth. The spore concentration approximated 550,000 spores per cc. as determined with a hemocytometer. The natural clonal lines were maintained by single-spore transfers and reisolation from inoculated plants. The method has given consistent results quite comparable to those in field plots used as supplementary tests without the hazards of other seedling parasite and environmental conditions over which there was no control. The tests were used in studies of races of the organism, soil and temperature relations, genetics of inheritance of resistance, and development of resistant varieties.

Radioisotope tests of translocation in artificially infected Pinus monticola Doug. HUBERT, ERNEST E. AND WILLIAM K. FERRELL. The University of Idaho is conducting studies to determine the cause of a new damaging disease of western white pine known as pole blight. Symptoms of this disease are appearing on 60,000 acres of pole size timber in northern Idaho, western Montana and British Columbia and many trees are dying. As part of these studies, under an AEC contract, tests are being conducted to determine the effect on translocation in the tree of certain fungi isolated from pole blighted trees. Radioactive phosphorus is being introduced into the xylem and the phloem to determine whether the bark lesions resulting from artificial inoculations cause any changes in translocation or absorption of the introduced solutions. The first series of tests are being conducted using cultures of *Leptographium* sp. as the inoculum on root, trunk, and branch tissues of small saplings in the greenhouse. Some evidence based on preliminary results indicates that bark lesions on the trunk seriously obstruct the downward movement of P32 in the phloem. Similar studies are planned using pine saplings artificially infected with *Armillaria mellea.*

The influence of western X little cherry virus upon seed size and viability. JONES, LEO E. AND J. A. MILBRATH. In 1950 and 1951, studies were made of morphological variations associated with the presence of western X little cherry virus in Bing, Royal Ann, Lambert, and Montmorency cherries growing near The Dalles, Oregon. The average weight per air-dried seed from fruit of healthy trees of all varieties was .047 grams. Fruits from each diseased tree were segregated into 3 types on the basis of fruit size and color. In these varieties, as the severity of virus symptoms in the fruit increased, the average weight of the seed decreased. The average weight per air-dried seed from each of the 3 types of fruits on diseased trees of all varieties was: 1) from "normal" appearing fruit—.047 grams, 2) from intermediate fruit—.030 grams, and 3) from severe little cherry fruit—.018 grams. Germination trials were made in the greenhouse with seed of the varieties and types given above. The average germination percentages of all varieties were: from healthy trees—14.5 per cent; from diseased trees 1) "normal"—15.9 per cent, 2) intermediate—1.6 per cent, and 3) severe little cherry—none.

An unreported Phytophthora rot of Swede turnips. JONES, WALTER. During the winters of 1951 and 1952 a storage rot of Swede turnips caused by a *Phytophthora* sp. similar to *Phytophthora megasperma* Drechsler in morphological and growth temperature characteristics was found in British Columbia. A characteristic external symptom was a "washing out" of the normal purplish color. The diseased flesh was soft and resilient and typically grayish in color; when pressed firmly a clear liquid oozed out. The fungus has proved pathogenic to seedlings of turnip, cauliflower, and cabbage, 4 varieties of Swede turnips, and to carrots and potatoes. The optimum temperature growth rate of the fungus is approximately 25° C., the minimum slightly below 5° C., and the maximum aproximately 30° C. When sections of cellophane cultures were placed in distilled water at different temperatures and observations made within 3 days, the optimum temperatures for sporangial development and organs of fusion were 20° C. and 24° C. respectively. No sporangia or organs of fusion developed at 5°, 10°, or 30° C., while at 24° C. only organs of fusion were produced. Since Brassica seedlings were readily infected artificially it appears that seed treatment should be helpful as a control measure.

A root rot tolerant lima bean. KENDRICK, J. B., JR., AND R. W. ALLARD. Root rot of Lima bean in the south coastal plain of California is incited principally by *Rhizoctonia solani* and *Fusarium solani* f. *phaseoli.* During the past 4 years varieties and breeding stock selections have been tested for their relative susceptibility to root rot in areas where heavy losses in Lima bean production due to a high incidence of root rot were known to occur. One of the breeding selections, designated L-4, has been outstanding in these tests with respect to root rot tolerance. Although infection of the root usually takes place, the invasion of the fungus is largely superficial and limited causing no extensive decay of the hypocotyl. Lines from single plant selections of L-4 show some difference in their susceptibility indicating that a certain amount of heterozygosity for tolerance still exists within the selection. The plant grows vigorously producing long runners not unlike the commercial variety, Ventura. The seed is a calico type with red irregular splotches occurring on a white background. This line is currently being used as the root rot tolerant parent in a breeding program designed to transfer the tolerance to the more susceptible green processing type Lima beans.

Influence of variety, temperature, and fungus strains upon Verticillium wilt of pepper. KENDRICK, J. B., JR., AND JOHN T. MIDDLETON. A characteristic symptom of Verticillium wilt of pepper incited by *Verticillium albo-atrum* is stunting resulting from internode shortening. An index, calculated by comparing mean increment growth of infected and noninfected plants was used to evaluate disease severity as influenced by varieties, soil temperature, and fungus strains. Among the 7 varieties studied, Anaheim Chili and Hungarian Paprika showed the greatest injury from infection and Oakview Wonder the least. No commercial variety tested showed sufficient disease tolerance to be grown successfully in infested soil. Optimum temperatures for disease expression are between 25° and 30° C., for all varieties tested. Between 15° and 25° C. severe stunting occurred; at 35° C. only the 2 most susceptible varieties showed moderate expressions of severity, while other varieties had near normal growth. Isolates of *V. albo-atrum* from 12 different hosts exhibited a range of pathogenicity to pepper. Only the pepper isolate incited severe symptoms. Isolates from rose and Chrysanthemum produced moderately severe symptoms, one from strawberry induced mild stunting, those from potato and okra produced very mild symptoms; those from boysenberry, radish, stock, avocado, melon, and tomato were practically non-pathogenic to pepper.

Use of the Williams-Backus method for determination of virus concentration in plant tissue cultures. KOBEL, F. TMV-

infected tobacco stem sections were grown on an agar medium containing indolebutyric acid in concentrations of 100, 10, and 1 mg/l. After 2 weeks incubation the small, newly formed calluses were removed, and their virus concentrations determined by means of the spray drop procedure described by Williams and Backus (1949). This method was found very helpful when only small amounts of tissue were available. Counting of the virus particles was greatly facilitated by treating the electron microscope screens with serum albumin before spraying the virus suspension. This treatment makes the film surface hydrophilic and causes better spreading of the droplets. Indolebutyric acid in a concentration of 100 mg/l. reduces the virus concentration in the calluses to 9.5 per cent of that of the controls which were grown on 1 mg/l. indolebutyric acid. The concentration of 10 mg/l. caused a virus reduction to 32 per cent.

Ascorbic acid content as an indication of virus infection of potato tubers. LOCKE, S. B. Newton's indicator agar-method for determination of ascorbic acid content (i.e., reducing power) of potato tuber tissues was applied to 173 tubers comprising 7 lots of 3 varieties. Although the average reducing power of tuber tissues infected with leafroll virus was greater than that of healthy tubers of the same lot, individual tubers could not be classified as healthy or diseased on this basis. It is concluded that this method is of very limited value for the determination of virus infection in potato tubers.

Tests for the occurrence of virus yellows in table beet seed plants in northwestern Washington. MCLEAN, D. M. A virus disorder of red table beets annually causes severe loss in seed production in northwestern Washington. Stecklings planted for seed production in March show no symptoms until June or July. Older leaves of infected plants then become chlorotic, thickened, and brittle. Young leaves show no symptoms, and no vein-clearing or foliar patterns have been observed. Chlorotic leaves become orange-yellow to bronze with conspicuous reddish interveinal spots that enlarge, often coalesce and become necrotic. Green peach aphis were fed on infected seed plants and 10 were transferred to each of 100 healthy (Detroit Dark Red) beet seedlings. After several weeks, all inoculated plants showed a mild yellowing and a thickened, brittle consistency of the older leaves. Control plants remained free of symptoms. Roots of inoculated plants, when stored and again planted the following spring, produced plants showing the same leaf symptoms. Serological tests of 5 leaf samples from different inoculated plants were made by G. H. Coons at Beltsville, using anti-yellows serum obtained from the Laboratorium voor Bloembollenonderzoek, Lisse, Netherlands. All samples gave positive reactions. The evidence indicates the presence of a "mild" yellowing virus in beets in Washington and its probable close relationship to that causing virus yellows in Europe.

Etiology and control of snowmold of turf in eastern Washington. MEINERS, J. P. Snowmold of turf in eastern Washington appears to be caused by a combination of 2 organisms, *Fusarium nivale* and *Typhula itoana*, with the former usually dominant. As part of the National Cooperative Turf Fungicide Trials, the ability of 15 fungicides to control snowmold was tested. The fungicides were applied in early December to 5 × 10 ft. replicated plots on golf greens in Pullman and Spokane. In most cases the dosages used were lighter than those recommended by manufacturers for snowmold control. In Pullman, where snowmold was very severe (95.6 per cent in untreated plots) the efficacy of the fungicides in decreasing order was as follows: PMAS, Puraturf, Calochlor, Cadminate, Calocure. Tact-C-Lect, Puarturf GG, Crag 531, C & C 1025, Special Semesan, Puraturf 177, Orthocide 406, Tersan, Chloranil (Spergon), and Actidione. Only the first 3 gave practical control. In Spokane, where snowmold infection was light (18.1 per cent in untreated plots) PMAS, Puraturf and Calochlor also gave adequate control as did also Tersan and Chloranil. Method of application (wet vs. dry) had no effect on degree of control obtained except in case of Cadminate where the dry method proved superior.

Strawberry virus complexes. MELLOR, FRANCES C. AND R. E. FITZPATRICK. The presence of a latent virus in healthy appearing British Sovereign and Marshall strawberry plants was demonstrated when these varieties were indexed with *Fragaria vesca*. This virus appears to exist in numerous intergrading strains. Its properties are the same as those of the non-persistent virus strains associated with Marshall yellows (xanthosis) and it is concluded that the two are identical. When Royal Sovereign strawberry plants were inoculated with these viruses alone no definite symptoms developed, but when the persistent component virus of Marshall yellows was included, the plants developed typical yellow-edge. It is apparent that the group of latent and non-persistent virus strains found in British Sovereign and Marshall is analogous to, if not identical with, Virus I described by Prentice and Harris, and that the persistent component of Marshall yellows is analogous to Prentice's Virus 2.

Curly-top virus induces potato green dwarf. MENZIES, J. D. AND N. J. GIDDINGS. Potato green dwarf has been reported from eastern Oregon and from central and eastern Washington. These are areas where the curly-top disease is very prevalent in susceptible hosts such as beet and tomato. The green dwarf disease is comparatively rare in commercial potato fields and is most often found near the margins. Experimental potato plantings made adjacent to plots of susceptible sugar beets, where curly-top infection was encouraged, have shown as much as 34 per cent Green Dwarf. The same symptoms were induced in healthy, greenhouse grown tomato stocks after they were grafted with either scions from field grown tomatoes showing curly-top symptoms or scions from green dwarf potatoes. Curly-top virus has been recovered from several of the green dwarf potato plants and from several of the tomato plants that had served as stocks for scions from the green dwarf plants. Results indicated that the curly-top virus concentration was relatively low in the potato. The recovered virus has been used to make many inoculations into potato, tomato, sugar beet, and other plants. A high percentage of infection was obtained and the symptoms were typical of curly-top.

Pythium seed decay and seedling blight of Pisum sativum. MIDDLETON, JOHN T. A study of the pathogenicity of 22 *Pythium* spp. to pea reveals that seed decay is incited primarily by *P. aphanidermatum*, *P. debaryanum*, *P. irregulare*, and *P. ultimum*. Seedling blight is incited by the same species and *P. oligandrum*, *P. paroecandrum*, *P. spinosum*, and *P. splendens*. Seed decay is initiated upon rupture of the seed coat and invasion of the embryo. The fungi usually invade the cotyledons and plumule and rarely the hypocotyl and radicle. Seedling blight develops as the plant grows and is generally first manifest as a decay of the epicotyl and later as a root rot. Under the conditions of soil moisture employed the amount of seed decay and seedling blight varied with the variety of pea, the quantity and virulence of the fungi, the depth of seeding, and soil temperature. Epicotyl infection occurs both before and after emergence and may either kill the young shoot or cause a partial cortical stem necrosis. Stem lesions often extend several inches above the soil and are responsible for unilateral wilting and leaf chlorosis. Stem infections are frequently initiated at the soil level and may not be associated with subterranean decay. Aerial infections develop rapidly through growth of the fungus within the stem cavity and vascular bundles.

Variations in potato calico caused by strains of alfalfa mosaic. MILBRATH, J. A. Potato calico has been known in Oregon since 1927 and until recently was considered to be caused by a strain of alfalfa mosaic. In 1946, a necrotic dwarfing strain was found in potatoes, and subsequent studies have shown this to be distinct from the calico

noted previously. This strain, called calico dwarf, becomes systemic in bountiful beans, causing a bright golden mottle and causes a bright golden chlorosis of many of its host plants. It causes severe necrosis without conspicuous chlorosis on potato. Recent studies with collections from various parts of the state indicate many variations in symptom behavior sufficiently significant to indicate strain differences. Therefore, it can be assumed that frequent mutations or changes are occurring in alfalfa mosaic which may account for the numerous papers on potatoes and legume viruses related to alfalfa mosaic.

Relation of temperature to persistence of strawberry yellows virus complex in the strawberry aphid. MILLER, P. W. Investigations on the relation of temperature to the persistence of strawberry yellows virus complex in the strawberry aphid (*Capitophorus fragaefolii* Ckll.) show that the components of the yellows complex persist in the aphid's body 3 to 4 days longer at cool temperatures (40° F.) than at relatively warm temperatures (70° F.). Not only was this true of the persistence component but also of the so-called non-persistent component which at room temperature generally dies out of the body of the aphid after about 6 hours of fasting. Futhermore, the aphids lived correspondingly longer without food at cool than at relatively warm temperatures. It is concluded that henceforth temperature will have to be taken into consideration in determining the limits of persistence of the various components in the strawberry aphid's body.

A new fungus disease of orchid seedlings in Hawaii. MURAKISHI, HARRY H. Root rot and wilt of *Cattleya, Dendrobium, Phalaenopsis,* and *Vanda* seedlings occur frequently during rainy periods. Isolations made from water-soaked and blackened lesions on roots and crowns of affected seedlings and older plants consistently yielded a *Fusarium*. Isolates from infected roots of *Cattleya, Dendrobium,* and *Vanda* seedlings and from adult *Oncidium* and *Dendrobium* plants caused rotting of one or more roots on 32 to 100 per cent of *Cattleya, Phalaenopsis,* and *Vanda* seedlings. The same isolates were less pathogenic (0-70 per cent root rot) on *Dendrobium*. On 3-year-old *Cattleya, Dendrobium,* and *Vanda* plants, wilt and root rot were also caused by a *Fusarium* from *Vanda* seedlings while root rot and wilt of *Dendrobium* and moderate root rot of *Cattleya* were produced by an isolate from an adult *Dendrobium* plant. A root rot and wilt of *Vanilla* (*Orchidaceae*) caused by a *Fusarium* is reported to occur in Puerto Rico. When rooted *Vanilla* cuttings were inoculated with 4 orchid *Fusarium* isolates, no root rot or wilt resulted although the plants were maintained in infested medium for over 6 months. Satisfactory control of the disease on *Vanda* seedlings was obtained by soaking the plants and pot in 1-2,000 Arasan or Tersan suspension.

Two new virus diseases in Hawaii. MURAKISHI, HARRY H. Two distinctly different virus diseases, one of Cattleya orchids and the other of *Desmodium canum*, Kaimi (Spanish) clover, have recently been found in Hawaii. Symptoms of the first named are stunting, distortion, and appearance of reddish-brown to blackish pitted areas and sunken streaks in the leaves and pseudo-bulbs. Transmission was obtained by leaf tissue grafting (1 out of 1) and by leaf rubbing (3 out of 5) in 20 to 30 days after inoculation. Sub-inoculations from infected test plants resulted in 4 out of 9 and 4 out of 5 transmissions using respectively tissue grafting and leaf rubbing methods. The second virus which causes a mosaic and severely reduces the yield of Kaimi clover is juice- and aphid- (*Myzus persicae*) transmissible to *Phaseolus vulgaris, P. lunatus, Crotalaria* spp., *Vigna sinensis, Soja max,* and *Trifolium subterraneum*, but not to *Pisum sativum, Lathyrus odoratus, Melilotus alba, M. officinalis, Medicago sativa, Trifolium pratense,* and *T. repens*. There was no evidence of seed transmission when 291 *D. purpureum* and 559 Bountiful beans were grown from seeds produced on infected plants. The virus *in vitro* is inactivated at 60° C., has a dilution endpoint of 1-2,000 and remains infective (*in vitro*) for 48 hours.

Strawberry root anatomy with special reference to black root rot. NELSON, PAUL E., AND STEPHEN WILHELM. The main roots of healthy strawberry plants and their prominent branches are perennial. These roots develop extensive secondary tissues from vascular and cork cambiums. The ultimate root branchlets generally lack the ability to produce secondary growth, and appear to be seasonal or at best annual. They possibly die naturally from degeneration of the primary phloem, or from infections by fungi. *Rhizophagus* sp. and mycorrhizal appearing types of Rhizoctonia are some of the suspected pathogens involved. Successive crops of seasonal (non-cambial) roots are produced yearly from the perennial root structure. Wood may become heartwood in the third year, as indicated by some plugging of vessels and by loss of ability of ray cells to store starch. A considerable portion of the large adventitious roots of plants affected with black root rot die before going into the second year type of development. The outer root tissues die first while the xylem continues to function for a longer time. Non-cambial (feeder) root formation from the surviving second year roots generally is poor.

The inhibition of tobacco mosaic symptoms by application of certain plant hormones. NICHOLS, CARL W. Young tobacco plants were sprayed daily with aqueous solutions of naphthaleneacetic acid at a concentration of 10 mg/l and indolebutyric acid at concentrations of 100 and 500 mg/l. Control plants were sprayed with distilled water. The naphthaleneacetic acid-sprayed plants developed tobacco mosaic symptoms 12 to 17 days after inoculation, the indolebutyric acid-sprayed plants in 13 to 30 days, and the distilled water-sprayed plants in 5 to 9 days. Symptoms were milder on the hormone-sprayed plants than on the water-sprayed plants. The plants treated with naphthaleneacetic acid (10 mg/l) and with indolebutyric acid (500 mg/l) were considerably stunted. Blister-like swellings on the stems and petioles and varying amounts of downward rolling of the leaves occurred on plants treated with both compounds. Fresh weights of the tops of healthy and infected plants sprayed with indolebutyric acid at a concentration of 100 mg/l were slightly higher than those of corresponding distilled water-sprayed plants.

Hot water treatments of some stone fruit viruses. NICHOLS, CARL W. AND GEORGE NYLAND. Virus infected cherry and peach budwood was treated at various times and temperatures in a hot water bath. After treatment, the buds were placed on mahaleb or Lovell seedlings in the nursery row. The necrotic rusty mottle virus was not inactivated in budwood from an infected mazzard seedling when treated at temperatures and times ranging from 38° C. for 8 hours, to 50° C. for 25 minutes. Peach budwood was treated at 48° C. for 5 and 10 minutes, 50° C. for 5 and 10 minutes, and 52° C. for 5 minutes. Inactivation of the yellow leaf roll virus varied from 4 per cent in the untreated checks to 56 per cent in the buds treated at 50° C. for 10 minutes. The yellow bud mosaic and Muir peach dwarf viruses were not inactivated in any of the treatments. Although 60 to 90 per cent of treated and untreated willow twig-infected buds "took," only 7 to 30 per cent of the buds grew in each treatment and no buds grew in the untreated checks. As yet, no symptoms have occurred on the growth from the treated willow twig-infected buds.

Separation of sour cherry yellows and ringspot viruses by passage through the seeds of Stockton Morello. NYLAND, GEORGE. Seed from 5 different sources of Stockton Morello known to be carrying sour cherry yellows and ringspot viruses was collected during the 1949 season and planted in 1950. Many seedlings showed symptoms resembling sour cherry yellows the first growing season, and additional ones expressed symptoms the following year. Thirty-five seedlings which showed yellows-like symptoms were indexed on Shirofugen and only 11 were positive for ring-spot, 9 gave a severe reaction, and 2 a very mild reaction. Buds from the other 24 seedlings did not induce gumming or necrosis on Shirofugen. Five seedlings that indexed negative for ringspot were budded to Lovell peach seedlings and

Montmorency variety of sour cherry. No recognizable symptoms have been produced on peach or Montmorency within 1 year. The very limited data so far available indicate that the viruses associated with the sour cherry yellows-like symptoms and ringspot will pass through the seeds of Stockton Morello independently of each other and that this method might be useful in obtaining pure cultures of seed-transmissible viruses and virus strains.

Host range and epidemiology of the cereal yellow-dwarf virus in California. OSWALD, JOHN W. AND BYRON R. HOUSTON. The yellow-dwarf disease of cereals which was epidemic in 1951 in California, was equally as severe in 1952 in late-planted grain. Five grass-infesting aphis species may transmit this newly-described cereal virus. Damage is directly proportional to the age of the grain when infected. Of 40 grass species tested with infective aphids, 19 exhibit symptoms of yellow-dwarf including *Bromus* sp., *Avena fatua*, *Hordeum* sp., *Festuca* sp., and *Phalaris* sp. Five grasses proved to be symptomless carriers. In 1950-51 continuous winter rains were favorable to both a rank growth of grasses and large populations of grass-infesting aphids. Rains also delayed planting of cereals until after March 1. A long drought followed, bringing about a rapid drying of grasses which forced aphids from these into the young cereal fields about March 20. This accounts for the sudden outbreak of yellow-dwarf in mid-April of 1951. Conditions in 1951-52 also favored grass and aphid development but most grain could be planted in December and January. Rains through March kept wild grasses green until mid-April when heavy aphid flights occurred. As a result only those fields planted after March 1 were severely damaged.

Effects of certain mercurial fungicides on gladiolus plants. PALM, ELMER T. Phytocidal effects of pre-planting corm treatments with several mercurial fungicides on Picardy gladiolus plants were determined in both field and greenhouse plots. Higher concentrations of all fungicides tested delayed emergence and flowering, resulting in the production of fewer flowers and smaller corms. Total emergence and total number of corms produced were reduced by immersing corms for 15 minutes in a suspension containing 8 lbs. of New Improved Ceresan in 100 gal. of water. Immersing corms for 15 and 45 minutes in a solution containing mercuric chloride (1:1000) resulted in the production of plants with narrow leaves. New Improved Ceresan, when used in the treatment recommended for controlling Fusarium and Sclerotinia corm rots under Oregon conditions (30-second immersion in a suspension containing 2 lbs. of New Improved Ceresan in 100 gal. of water), delayed both emergence and flowering 1 day but otherwise was not detrimental to gladiolus plants. When corms were immersed 15 min. in a similar suspension of New Improved Ceresan, emergence was delayed 5 days, flowering was delayed 3 days, total flowering was reduced 22 per cent, and resultant corms were approximately 5 per cent larger than those in an untreated series.

Crystalline inclusions produced by red clover vein mosaic virus. PORTER, C. A. AND F. P. MCWHORTER. *Vicia faba* L. plants inoculated with either a strain of red clover vein mosaic virus from Oregon or with a type strain from Wisconsin developed specific cytoplasmic inclusions in epidermal cells of leaves. The inclusions occur most abundantly in epidermal cells over hyaline veins and adjacent to necrotic areas. The shape and size of the inclusions are variable. They range from aggregates of rounded bodies 2μ in diameter to nearly perfect hexagonal prisms 20 x 9μ. The most common form is a hexagonal prism with unequal sides and rounded corners. The inclusions are proteinaceous in nature as evidenced by a positive ninhydrin reaction and by iodine staining. The presence of ribonucleic acid (RNA) in the crystals is indicated by their staining intensely with pyronin. When the crystals are hydrolized with trichloracetic acid to remove the RNA, the staining is reduced to a trace thus indicating that the coloration is truly due to RNA. The absence of desoxyribonucleic acid (DNA) in the crystals is indicated by a negative Feulgen reaction . They do not stain with trypan blue as do the inclusions of bean yellow mosaic and etch viruses. They appear to be largely protein as indicated above and by tests that prove the absence of starch and fats within the crystals.

Infection experiments with the fungus causing the sooty blotch disease of Vitis californica Benth. PURDY, LAURENCE H. JR., AND WM. B. HEWITT. The lower and upper surfaces of leaves of *V. californica* were inoculated with drops of surface mycelium and conidia from monoascosporic cultures on P. D. A. of the species Mycosphaerella which causes sooty blotch. Twenty-four of the 50 leaves inoculated on the lower surface and only 2 of the 62 leaves inoculated on the upper surface became infected. Since stomata occur only on the lower surface of grape, these results indicated that infection took place through the stomata. Varieties of *V. vinifera* L.: Sultana, Rish Baba, and Thompson Seedless, were not susceptible, while all plants of *V. californica* inoculated at the same time became infected. A study of herbarium specimens of a similar disease on Sultana grape leaves from Pakistan and South Africa show that the leaf spot disease from these 2 localities is caused by the same fungus. When compared with the California leaf spot fungus only slight morphological differences in conidia and conidiophores were observed. The similarity of these fungi indicate that they are closely related. Differences in morphology are not considered great enough to designate them as different species even though the California fungus does not infect the variety Sultana.

Infection of lettuce by Sclerotinia sclerotiorum (Lib.) de Bary. PURDY, LAURENCE H., JR. AND R. G. GROGAN. The role of non-living organic matter in the infection of lettuce by *Sclerotinia sclerotiorum* (Lib.) de Bary has not been emphasized. It was suspected that infection of lettuce by mycelium from germinating sclerotia occurred only when non-living organic matter was present because no infection resulted when germinating sclerotia alone were in contact with the stem. Infection occurred in 4 days using vegetative mycelium on potato dextrose agar blocks when they were placed at the soil surface in contact with the stem. Under similar conditions no infection resulted when bare sclerotia were used if all dead lower leaves of the plant were removed prior to inoculation. That non-living organic matter was necessary to initiate infection was also indicated in a laboratory moist chamber because mycelium from germinating sclerotia adhered to the stem but failed to infect. In the greenhouse, infection occurred in 5 days when sclerotia were placed about ¼ in. from the stem and covered with dead leaves that were also allowed to touch the stem.

Factors affecting the rate of decay of old-growth Douglas fir by Fomes pini. ROTH, LEWIS F. Estimates of future volumes of old-growth Douglas fir are difficult because of unpredictable volume reduction by *Fomes pini* decay. Stand deterioration rate is determined by the number of individuals infected and by the rate of spread within individuals. Experiments evaluating decay extent within individual trees determined influences of: number of infections per tree, decay capacity of fungus isolates, inherent wood resistance. Time of infection and decay interval of test trees are unknown. Frequency of infection was determined by plating, in sequence, combinations of cultures isolated at intervals along the bole. Cultures from identical mycelia grew homogeneously together; those from different mycelia were incompatible. Where incompatibilities appeared multiple mycelia were indicated, hence multiple infections. Of 73 trees 70 per cent had single infections, 25 per cent had 2, and 5 per cent more than 2. The respective decay extents were 84, 106, and 146 ft. Volumes lost were 2,880, 3,780, 4,330 bd. ft. There was a significant positive correlation between colony diameter and decay. Decay rate of wood blocks was positively correlated with fungus growth (computed t: 3.06, table value: 2.75). Inner and outer heartwood and sapwood from 11 trees showing extremes of decay were tested for decay resistance, using the Leutritz method. Hu-

midity was 80 per cent, temperature 80° F. Sapwood decayed more rapidly than heartwood. Significant differences among trees appeared correlated with lineal decay extent.

Effect of radioactive phosphorus on symptoms and virus content of mosaic tobacco plants. SCHLEGAL, DAVID E., C. C. DELWICHE, AND A. H. GOLD. Turkish tobacco seedlings inoculated with tobacco mosaic virus (TMV) were placed in culture solutions containing various levels at P^{32} up to 12.0 mc. per 1,800 ml. of culture solution and allowed to remain for 10 days, whereupon they were harvested and assayed for TMV. At the end of the experimental period young leaves of infected plants in radioactive solutions showed a decided suppression of TMV symptoms above concentrations of 3.5 mc. per 1,800 ml. The actual yield of virus in the younger leaves was lower than the controls in solutions containing as little as 0.4 mc. per 1,800 ml., and the virus yield was roughly inversely proportional to the log. of phosphorus concentration. Above 3.5 mc. per 1,800 ml. plants showed decided radiation damage characterized by a cessation of cell division, resulting in deformed roots and suppressed apical development. Cell elongation was apparently less seriously affected by com parison, since expansion of older leaves appeared to be normal. Older, inoculated leaves showed little or no radiation damage and no significant reduction of virus yield. In general, no large differential effect of radiation on virus formation versus new plant tissue formation was demonstrated.

Fungitoxicity investigations using a novel spore germination technique. SHAFER, WILLIAM B. This bioassay method consists of placing 0.2 ml. of each of a series of concentrations of aqueous dispersions or solutions of the test compound in individual microbeakers. To this chemical solution or dispersion is then added another 0.2 ml. of a carefully standardized fungus spore suspension which contains a known number of spores. The microbeakers are then placed in a controlled temperature chamber for germination of the fungus spores. After a germination period of 16 hours, drops of the spore suspension from each microbeaker are examined microscopically and the percentage of spores failing to germinate recorded. The data obtained may be examined statistically in the usual manner for determination of the LD-95 and LD-50 points.

A quantitative electrophoretic study of competition between two strains of tobacco mosaic virus. I. The inoculated mature leaf. SIEGEL, ALBERT AND MORRIS COHEN. The fact that different viruses may have different electrophoretic mobilities makes possible a direct quantitative approach to the hitherto difficult problem of the nature of competition between viruses. The electrophoretic method has been applied to the study of the relative rates of multiplication of 2 strains of tobacco mosaic virus in the same plant. The virus strains studied were the common strain and a derivative "mild" strain, which produces a mild mosaic in *Nicotiana tabacum* and which has a markedly lower electrophoretic mobility than the common strain. Mature leaves of 18-in. tall greenhouse grown tobacco plants in 6-in. pots were simultaneously inoculated with the 2 strains in various ratios. After 2 weeks of infection, the ratio of the 2 strains of virus present in these leaves was the same as the ratio of the 2 strains in the inoculum. For the first 2 weeks after inoculation of matured leaves with equal parts of the 2 strains, both strains increased at the same rate. From the second to the fourth week after inoculation the common strain increased at a slightly greater rate than the mild strain.

Composition of crystalline inclusion bodies of tobacco mosaic virus. STEERE, RUSSELL L. AND ROBLEY C. WILLIAMS. Crystalline inclusion bodies have been removed from hair cells of Turkish tobacco plants infected with tobacco mosaic virus (TMV). A freeze-drying method is employed, the steps of which are: strips of epidermis with numerous hair cells containing crystalline inclusions are rapidly frozen in liquid nitrogen; the hairs are removed and vacuum dried while maintained at a temperature of −35° C.; crystals are then teased from the frozen-dried cells. The crystals can be manipulated intact with the end of a fine needle and their dimensions measured. They dissolve readily in distilled water and can then be examined with the electron microscope or tested for infectivity. Electron microscopic observations show the frozen-dried crystals to be composed of TMV rods comprising 25-40 per cent of the total volume, the remainder being essentially free of particulate matter. Virus from dissolved crystals has the same range of infectivity at equal particle concentrations as that obtained with diluted crude juice from infected plants.

Control of celery late blight. VAUGHAN, EDWARD K. *Septoria apii* is abundant in the soil of all major celery-growing sections of western Oregon. Late blight is present every year and frequently causes severe losses, particularly in celery grown for harvesting after the start of the fall rains. Spray and dust trials over the past 4 years have shown that the losses can be almost entirely prevented by timely application of fungicides. Ziram (1½ lbs./100 gal. or 10 per cent dust) and tribasic copper sulphate (6 lbs./100 gal.) have given best control without evidence of phytotoxicity. During the dry season dusts and sprays applied at 14-day intervals have given comparable control. After the beginning of the fall rains only spraying gives satisfactory control and the interval between applications must be shortened to 7 to 10 days.

Symptoms in sweet cherry produced by western X-disease virus from peach. WADLEY, BRYCE N. Western X-disease virus from peach trees produced rosetting stunting of leaves and enlargement of stipules on mazzard cherry seedling trees. Of 50 cherry trees inoculated in March, 1951, in the greenhouse by grafting with infected peach buds or scions or by approach grafting them to infected peach trees, 18 developed typical symptoms in new growth following dormancy; 12 trees died, presumably from the effects of the virus, before new growth was produced. Similar leaf and stipule symptoms are found on naturally infected sweet and sour cherry trees in Utah. Western X-disease virus from peach trees produced wilt in Napolean cherry on Mahaleb understock when the cherry trees were inoculated by the approach graft method. Approach grafts were more efficient than bud or scion grafts for transferring western X-disease virus from peach to sweet cherry. Transfers of western X-disease virus are more readily made from sweet cherry to peach than from peach to sweet cherry.

Year-to-year variation in symptom expression of western X-disease on peaches in Utah. WADLEY, BRYCE N. In Utah western X-disease symptoms on peaches are much milder in certain years than in others. Symptoms in 1942, 1950, and 1951 were generally much later appearing than usual and were confined to smaller portions of the infected trees. Frequently, symptoms were found only on a few small branches or twigs. Some trees, known from previous records to be infected, failed to develop recognizable symptoms. Severe western X-disease symptoms were observed in 1939, 1940, 1941, 1948, and 1949. No data are available concerning the severity of western X-disease from 1943 to 1947. Weather Bureau Climatalogical Data for 5 points in the Utah fruit-growing area (Logan, Ogden, Farmington, Salt Lake, and Provo) show that mean temperatures during May and June of the 3 years with mild symptoms were 3.4°, 4.6°, and 2.6° F. below normal, respectively. Mean temperatures for May and June of the 5 years with severe symptoms were 1.0°, 9.9°, 4.7°, 2.4°, and 2.1° F. above normal, respectively. Relatively high temperatures during the growing season appear to be necessary for development of severe symptoms of western X-disease on peach in Utah.

Solanum sarachoides, an important weed host to Verticillium alboatrum. WILHELM, STEPHEN AND HAROLD E. THOMAS. *Solanum sarachoides* Sendt., a South American nightshade now widely distributed in Western United States, is an important weed host to *Verticillium albo-atrum.* Infected plants, despite extensive infection of the xylem by the fungus, are symptomless, execpt for occasional

reddish-brown vascular discoloration in older plants. Strawberry failures due to the Verticillium disease have been traced to the build-up of inoculum by this weed. In tests, nightshade isolates from 3 localities have been severely pathogenic for strawberry, but have been non-pathogenic for tomato. Occasionally tomato may be infected, if roots are dipped into a spore suspension, but even though infection results, no perceptible injury to tomato is observed. In this respect nightshade isolates are similar to most isolates from potato tested thus far.

Development of bud failure in the Jordanolo variety of almond. WILSON, E. E. The Jordanolo almond (Nonpareil × Harriott) is affected by a bud failure disorder which is probably non-transmissible by grafting but is scion-perpetuated and is increased by vegetative propagation. Trees produced from a given source of bud-wood exhibit wide differences in rates and severity of symptom development. Some develop symptoms 2 seasons after planting, others not until 5 or 6 years later. In one lot, for example, 10 per cent of the trees developed symptoms in 3 years and an additional 40 per cent developed symptoms in 5 years. Certain trees exhibit bud failure in a few branches only; others exhibit it in all branches. Young trees develop symptoms more rapidly than mature ones. In fact, symptom development may become more or less static after 12 to 15 years. Consequently, trees which exhibit a mild form of the disorder at maturity may continue for some years to exhibit it in a mild form. However, if, mature trees are forced into vigorous growth by severe pruning, bud failure develops as rapidly as on young trees. Buds taken from certain mature Jordanolo trees with no recognizable evidence of bud failure carried the disorder, as did buds from symptomless branches of affected trees.

Relative importance of seed and soil borne infection in damping-off of garden beets. YALE, JOHN W. AND EDWARD K. VAUGHAN. Isolation and soil inoculation studies have shown damping-off in table beets in the Willamette Valley of Oregon to be caused primarily by *Pythium ultimum* and *Fusarium* spp. *Fusarium* spp. as well as other fungi were shown to be present in the seed balls. Disease-free beet seed was obtained by testing in water at 55°C. for 30 min. In an experiment to determine the relative importance of seed and soil-borne organisms, commercial beet seed from 3 sources was grown as follows: (a) disease-free seed in sterile soil, (b) disease-free seed in infested soil, (c) untreated seed in sterile soil, and (d) untreated seed in infested soil. Results indicated that seed-borne organisms were relatively unimportant as causes of damping-off and that seedborne pathogens do not move rapidly from diseased to healthy seedlings in sterile soil.

Guttation due to leaf pressure favors fungus infections. YARWOOD, C. E. When young detached bean leaves were floated with their upper or lower surfaces on water in a humid chamber, water of guttation usually formed on the opposite leaf surface exposed to the air. With medium-aged leaves treated as above, there was little guttation, but it occurred on the abaxial exposed leaf surface when a portion of the opposite surface was rubbed with carborundum or when a portion of the leaf was cut away. It also occurred when a short piece of the petiole was placed in water. Guttation amounted to as much as 6 mg./cm^2 in a 12 hour period. On leaves rubbed with carborundum, guttation occurred on leaves on 0.1 and 1 per cent sucrose solution, but usually not on 10 per cent sucrose. Of 8 species tested, guttation due to leaf pressure occurred only on bean, (*Phaseolus vulgaris*), broad bean (*Vicia faba*), and tobacco (*Nicotiana tabacum*). When the test bean leaves were inoculated with dry spores of *Uromyces phaseoli*, or *Colletotrichum lindemuthianum*, and broad bean leaves with *Botrytis cinerea*, the resulting infection was positively correlated with the amount of guttation.

Latent period and generation time of tobacco mosaic virus. YARWOOD, C. E. Tobacco leaves were inoculated with tobacco mosaic virus, washed, dried, detached, incubated in dark moist chambers at 31° C., and sampled at intervals. Samples were assayed for virus by inoculating bean leaves with 1 per cent of the macerated tissue suspended in 0.5 per cent K_2HPO_4 solution, and counting the number of virus lesions produced. By this method virus could be detected at 10^{-6} of the concentration in systemically infected tobacco plants. Washing off unrubbed leaves freshly sprayed with virus removed 99.9 per cent of the deposited virus, but washing off leaves rubbed with virus removed only 94 per cent of the virus. Within the first hour after inoculation there was a decrease in the amount of virus recovered, at 1-7 hours after inoculation the amount of recovered virus remained approximately constant, and after 7 hours the amount of recovered virus approximately doubled each hour. This generation time was less with 30 per cent inoculum than with 0.1 per cent, was less on bean than on tobacco, and was decreased at temperatures above and below 31° C. When inoculated bean leaves were dipped in water for 1 hour within 8 hours after inoculation, the resulting infection was less than on untreated leaves.

Incipient crown gall infections in Mazzard cherry seedlings. YOUNG, ROY A. The development of crown gall on Mazzard cherry understock annually causes large losses to nurserymen. Attempts were made in 1949 and 1950 to reduce the incidence of crown gall on Mazzard cherry by dipping seedlings in various chemicals prior to lining out. More than 40 chemicals or combinations of chemicals were tested. None of the treatments significantly reduced the incidence of crown gall. Lack of significant differences indicated that seedlings might already be infected at time of treatment. Therefore seedlings from 3 different sources were tested for the presence of incipient infections. Seedlings from source A were root-pruned and planted directly in sterilized soil. After 6 months 26.8 per cent of the seedlings had developed crown gall. One half of the seedlings from sources B and C were root-pruned, then planted directly in sterilized soil, while the remaining seedlings were root-pruned, soaked in 15 per cent commercial Clorox for 1 minute, then planted in sterilized soil. After 6 months 10.6 and 11.0 per cent respectively of the untreated and treated seedlings from source B, and 8.2 and 6.9 per cent respectively of the untreated and treated seedlings from source C had developed crown gall.